AF/ #16 44/ lc

TRANSMITTAL FORM

(to be used for all correspondence after initial filing)

Application Number	09/643,277
Filing Date	August 22, 2000
First Named Inventor	Nelson, M. Bud
Art Unit	1644
Examiner Name	Saunders, D.A.
Attorney Docket Number	26983-50

Total Number of Pages in This Submission

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ENCLOSURES (*Check all that apply*)

✔ Fee Transmittal Form	☐ Drawing(s)	☐ After Allowance Communication to a Technology Center (TC)
✔ Fee Attached	☐ Licensing-related Papers	✔ Appeal Communication to Board of Appeals and Interferences
☐ Amendment/Reply	☐ Petition	☐ Appeal Communication to TC (Appeal Notice, Brief, Reply Brief)
☐ After Final	☐ Petition to Convert to a Provisional Application	☐ Proprietary Information
☐ Affidavits/declaration(s)	☐ Power of Attorney, Revocation Change of Correspondence Address	☐ Status Letter
☐ Extension of Time Request	☐ Terminal Disclaimer	✔ Other Enclosure(s) (please Identify below):
☐ Express Abandonment Request	☐ Request for Refund	Cert. of Mailing; Form PTO-2038; Return receipt post card.
☐ Information Disclosure Statement	☐ CD, Number of CD(s) _____	
☐ Certified Copy of Priority Document(s)	**Remarks**	
☐ Response to Missing Parts/ Incomplete Application	Appeal Communication = Appeal Brief	
☐ Response to Missing Parts under 37 CFR 1.52 or 1.53		

SIGNATURE OF APPLICANT, ATTORNEY, OR AGENT

Firm or Individual	N. Nicole Endejann Benesch, Friedlander, Coplan & Aronoff, LLP
Signature	*[signature]*
Date	October 9, 2003

CERTIFICATE OF TRANSMISSION/MAILING

Typed or printed	Christina R. Correll		
Signature	*[signature]* Christina R. Correll	Date	10/09/03

FEE TRANSMITTAL
for FY 2004

Effective 10/01/2003. Patent fees are subject to annual revision.

☑ Applicant claims small entity status. See 37 CFR 1.27

TOTAL AMOUNT OF PAYMENT	**($) 165**

Complete if Known

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METHOD OF PAYMENT *(check all that apply)*

☐ Check ☑ Credit card ☐ Money Order ☐ Other ☐ None

☑ Deposit Account:

Deposit Account Number	02-2051
Deposit Account Name	Benesch, Friedlander, C..

The Director is authorized to: *(check all that apply)*

☑ Charge fee(s) indicated below   ☑ Credit any overpayments

☑ Charge any additional fee(s) or any underpayment of fee(s)

☐ Charge fee(s) indicated below, **except for the filing fee** to the above-identified deposit account.

FEE CALCULATION

1. BASIC FILING FEE

Large Entity Fee Code	Fee ($)	Small Entity Fee Code	Fee ($)	Fee Description	Fee Paid
1001	770	2001	385	Utility filing fee	
1002	340	2002	170	Design filing fee	
1003	530	2003	265	Plant filing fee	
1004	770	2004	385	Reissue filing fee	
1005	160	2005	80	Provisional filing fee	
				SUBTOTAL (1)	**($)**

2. EXTRA CLAIM FEES FOR UTILITY AND REISSUE

	Extra Claims	Fee from below	Fee Paid
Total Claims	-20** =	X	=
Independent Claims	- 3** =	X	=
Multiple Dependent			=

Large Entity Fee Code	Fee ($)	Small Entity Fee Code	Fee ($)	Fee Description
1202	18	2202	9	Claims in excess of 20
1201	86	2201	43	Independent claims in excess of 3
1203	290	2203	145	Multiple dependent claim, if not paid
1204	86	2204	43	** Reissue independent claims over original patent
1205	18	2205	9	** Reissue claims in excess of 20 and over original patent

SUBTOTAL (2) **($)**

**or number previously paid, if greater; For Reissues, see above

FEE CALCULATION *(continued)*

3. ADDITIONAL FEES

Large Entity Fee Code	Fee ($)	Small Entity Fee Code	Fee ($)	Fee Description	Fee Paid
1051	130	2051	65	Surcharge - late filing fee or oath	
1052	50	2052	25	Surcharge - late provisional filing fee or cover sheet	
1053	130	1053	130	Non-English specification	
1812	2,520	1812	2,520	For filing a request for *ex parte* reexamination	
1804	920*	1804	920*	Requesting publication of SIR prior to Examiner action	
1805	1,840*	1805	1,840*	Requesting publication of SIR after Examiner action	
1251	110	2251	55	Extension for reply within first month	
1252	420	2252	210	Extension for reply within second month	
1253	950	2253	475	Extension for reply within third month	
1254	1,480	2254	740	Extension for reply within fourth month	
1255	2,010	2255	1,005	Extension for reply within fifth month	
1401	330	2401	165	Notice of Appeal	
1402	330	2402	165	Filing a brief in support of an appeal	165.00
1403	290	2403	145	Request for oral hearing	
1451	1,510	1451	1,510	Petition to institute a public use proceeding	
1452	110	2452	55	Petition to revive - unavoidable	
1453	1,330	2453	665	Petition to revive - unintentional	
1501	1,330	2501	665	Utility issue fee (or reissue)	
1502	480	2502	240	Design issue fee	
1503	640	2503	320	Plant issue fee	
1460	130	1460	130	Petitions to the Commissioner	
1807	50	1807	50	Processing fee under 37 CFR 1.17(q)	
1806	180	1806	180	Submission of Information Disclosure Stmt	
8021	40	8021	40	Recording each patent assignment per property (times number of properties)	
1809	770	2809	385	Filing a submission after final rejection (37 CFR 1.129(a))	
1810	770	2810	385	For each additional invention to be examined (37 CFR 1.129(b))	
1801	770	2801	385	Request for Continued Examination (RCE)	
1802	900	1802	900	Request for expedited examination of a design application	

Other fee (specify) _____

*Reduced by Basic Filing Fee Paid

SUBTOTAL (3) **($) 165.00**

SUBMITTED BY

(Complete (if applicable))

Name (Print/Type)	N. Nicole Endejann	Registration No. (Attorney/Agent)	50,229	Telephone	216-363-4504
Signature				Date	10/09/03

WARNING: Information on this form may become public. Credit card information should not be included on this form. Provide credit card information and authorization on PTO-2038.

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of: Nelson, M. Bud)
)
Serial No: 09/643,277   )
)
Filed: August 22, 2000   )
)
Title: Compositions, Assay Kits)
                  And Methods For Use   )
                  Related To A Disease   )
                  Condition Comprising   )
                  Multiple Sclerosis And/Or)
                  A Pro-MS Immune   )
                  Response   )
)
Art Unit: 1644   )
)
Examiner: Saunders, D.A.   )

Mail Stop Appeal Brief - Patents
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PO Box 1450
Alexandria, VA 22313-1450

APPEAL BRIEF

PATENT APPLICATION

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE
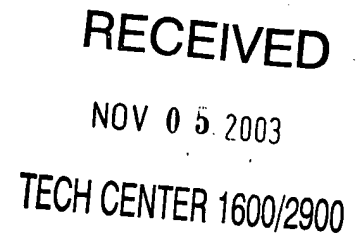
In re application:	Nelson, M. Bud	Art Group:	1644
Serial No.:	09/643,277	Examiner:	Saunders, David A.
Filing Date:	August 22, 2000	Docket No.:	26983-50
Title:	COMPOSITIONS, ASSAY KITS, AND METHODS FOR USE RELATED TO A DISEASE CONDITION COMPRISING MULTIPLE SCLEROSIS AND/OR A PRO-MS IMMUNE RESPONSE		

CERTIFICATE OF MAILING UNDER 37 CFR 1.8

I hereby certify that the below-identified papers are being deposited with the United States Postal Service with sufficient postage as "FIRST CLASS MAIL" on **October 9, 2003**, in an envelope addressed to: Mail Stop Appeal Briefs - Patents, Commissioner for Patents, Box 1450, Alexandria, VA 22313-1450.

Christina R. Correll
(type or print name of person mailing paper)

Christina R. Correll
Signature of person mailing paper

Items enclosed herewith:
1. Appeal Brief (9 pages in triplicate);
2. Transmittal Form;
3. Fee Transmittal Form;
4. Credit Card Payment Form PTO-2038; and
5. Return receipt postcard.

TABLE OF CONTENTS

TABLE OF AUTHORITIES

This is an appeal from the final rejection of all claims in the above-identified application (Ex. 1).

The fees required under 37 C.F.R. § 1.17 are dealt with in the accompanying Transmittal of Appeal Brief.

The brief is filed in triplicate (37 C.F.R. § 1.192(a)).
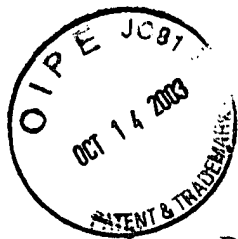
I. BIOCRYSTAL IS THE REAL PARTY IN INTEREST (37 C.F.R. § 1.192(c)(1))

BioCrystal is the Assignee of the subject application and is the real party in interest.

II. RELATED APPEALS AND INTERFERENCES (37 C.F.R. § 1.192(c)(2))

There are no related appeals or interferences.

III. STATUS OF CLAIMS (37 C.F.R. § 1.192(c)(3))

A. The status of the claims in the application are:

Claims 1-9, 18, 19, 29, 30, and 41-45 were cancelled.

Claims 10-17, 20-28, 31-40, and 46-62 are rejected under 35 U.S.C. § 112, second paragraph in the Advisory Action dated July 18, 2003.

B. Claims on appeal are:

Claims 10-17, 20-28, 31-40, and 46-62 (attached as Appendix "A").

IV. STATUS OF AMENDMENTS (37 C.F.R. § 1.192(c)(4))

The Examiner stated in the Advisory Action dated July 18, 2003 that Applicant's Amendment B has been entered.

V. SUMMARY OF INVENTION (37 C.F.R. § 1.192(c)(5))

The application is directed to methods of detecting and assaying markers which indicate a disease condition consisting of multiple sclerosis, a pro- multiple sclerosis immune response and a combination thereof.

In particular, the method is achieved because of a discovery that a combination of affinity ligands can be used in an immunoassay to generate one or more indicators that appear to differ in individuals having a disease condition selected from the group consisting of multiple sclerosis, a pro- multiple sclerosis immune response, and a combination thereof, as compared to a comparative values determined from healthy controls or in individuals having inflammatory diseases other than multiple sclerosis.

VI. ISSUES (37 C.F.R. § 1.192(c)(6))

Whether claims 10-17, 20-28, 31-40, and 46-62 are unpatentable under 35 U.S.C. § 112 (¶ 2) as being indefinite for failing to particularly point out and distinctly claim the subject matter which Applicant regards as his invention.

VII. GROUPING OF CLAIMS (37 C.F.R. § 1.192(c)(7))

Alleged Indefinite Terms	Grouping of Claims
Indicator	10-17, 20-28, 32-33, 40, and 54-62
Affinity Ligand Not Comprising a Detection Reagent	10-17, 20-28, 31-40, and 46-62

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VIII. <u>ARGUMENT</u>

A. THE CLAIMS PATENTABLY POINT OUT AND DISTINCTLY CLAIM THE INVENTION AND THE REJECTIONS UNDER 35 U.S.C. § 112 (¶ 2) SHOULD BE REVERSED

The Office has rejected claims 10-17, 20-28, 31-40 and 46-62 under 35 U.S.C. § 112, second paragraph as being indefinite. (Ex. 2, pg. 2). In particular, the Office alleges that the terms "indicator" and the "affinity ligand which does not comprise a detection reagent" render the claims confusing. (Ex. 2, pg. 2).

Under 35 U.S.C. § 112, second paragraph, a specification shall include claims "particularly pointing out and distinctly claiming the subject matter which the applicant regards as his invention." 35 U.S.C. § 112, (¶2). The standard for determining whether this requirement has been met is "whether those skilled in the art would understand what is claimed when the claim is read in light of the specification." *The Beachcombers, Int'l Inc. v. WildeWood Creative Prods. Inc.*, 31 F.3d 1154, 31 U.S.P.Q.2d 1653, 1656 (Fed. Cir. 1994) (quoting *Orthokinetics Inc. v. Safety Travel Chairs Inc.*, 806 F.2d 1565, 1576, 1 U.S.P.Q.2d 1081, 1088 (Fed. Cir. 1986)). "If the claims, read in light of the specification reasonably apprise those skilled in the art of the scope of the invention, § 112 demands no more." *Personalized Media Commun. LLC v. ITC*, 161 F.3d 696, 48 U.S.P.Q.2d 1880, 1888 (Fed. Cir. 1998) (quoting *Miles Lab., Inc. v. Shandon, Inc.*, 997 F.2d 870, 875, 27 U.S.P.Q.2d 1123, 1126 (Fed. Cir. 1993)). In other words, the claims must be sufficiently precise to allow a potential competitor to determine whether or not he is infringing. *Morton Int'l Inc. v. Cardinal Chem. Co.*, 5 F.3d 1464, 28 U.S.P.Q.2d 1190, 1195 (Fed. Cir. 1993).

1. Indicator

The Office has maintained the position that the claims are confusing as to what is the indicator. Specifically, in the first Office Action dated August 26, 2002, the Office asserted that it is unclear whether the indicator is (1) the same as the marker, or (2) a detectable entity, or (3) a nonphysical entity. (Ex. 3, pg. 3). In Amendment A and Response to Office Action, the Applicant clearly explained that the term "indicator" as used in the claims refers to markers meeting a condition. (Ex. 4, pgs. 6-7). For example, the condition includes the comparison between a reference value and a measured value of the marker.

The Office replied that "Applicant's response has clarified the record as to what he intends to claim by reciting 'indicator.'" (Ex. 5, pg. 2). However the Office continued that "For what applicant intends, the term –indication— would be more appropriate than 'indicator' in claims 10, 20, 32 and 54." (Ex. 5, pg. 2). The Office reasoned that the term "indication" is conventionally used in claim language for clinical diagnostic methods, in a concluding step that relates the presence/amount of a diagnostic marker to the presence/absence/stage/course of a disease condition, whereas the term "indicator" implies a chemical entity which provides a signal response in an assay. (Ex. 5, pg. 2).

While the Office may believe that one term may be more appropriate than another term, a quintessential tenet of patent law entitles the inventor to be his own lexicographer. While a term may not be given a meaning repugnant to the usual meaning of the term, a patentee may use a term in a manner contrary to or inconsistent with one or more of its ordinary meanings, and to the extent it differs from the conventional meaning, the patentee must clearly set forth the meaning in the specification. MPEP § 2173.05(a); *see also Beachcombers*, 31 U.S.P.Q.2d at

1656. Extrinsic evidence may not be used if the intrinsic evidence, namely the specification, unambiguously defines the claim language. *Personalized Media*, 48 U.S.P.Q.2d at 1889.

In the instant case, Applicant has clearly and consistently set forth the term "indicator." As used in the claims, and reasserted during the prosecution, an indicator is a marker meeting a condition, for example a comparison between a reference value and a measured value of the marker. (Ex. 1, pg. 15-16, 20-21, 26-27, 29-30). Such a meaning is not repugnant or even inconsistent with the ordinary meaning of the term. As defined in Webster's New College Dictionary, indicator is "a substance, as litmus or phenolphthalein, that indicates the presence, absence, or concentration of a substance or the degree of reaction between two or more substances by means of a characteristic change, esp. in color...." WEBSTER'S NEW COLLEGE DICTIONARY p. 564 (1995). The meaning of "indicator" as used by Applicant is encompassed by the ordinary meaning of the term. Moreover, Applicant has also clearly set for the meaning in the specification. (Ex. 1, pg. 15-16). Based upon the specification, one of ordinary skill in the art would understand the scope of the claims and be apprised of what Applicant regards as his invention. Thus, the rejection of claims 10-17, 20-28, 32-33, 40 and 54-62 under 35 USC § 112, second paragraph is improper.

2. The Affinity Ligand Not Comprising a Detection Reagent

The Office stated that claims 10, 20, 31, 46 and 54 remain confusing by failing to recite how the affinity ligand which does not comprise a detection reagent participates to form any sort of complex, to which the affinity ligand which comprises a detection reagent becomes bound. (Ex. 5, pg. 2). The Office reasoned that the claims per se must clearly define the relationship of all of the recited reagents, without which there is a gap in the claimed method steps. (Ex. 5, pg. 2).

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The public is entitled to know the scope of the claims, but must also look to the specification and prosecution history in such a determination. *See Texas Instrum. v. ITC*, 871 F.2d 1054, 10 U.S.P.Q.2d 1257, 1264 (Fed. Cir. 1989). It is not necessary that a claim recite each and every element needed for the practical utilization of the claimed subject matter. *Carl Zeiss Stinfung v. Renishaw plc*, 945 F.2d 1173, 20 U.S.P.Q.2d 1094, 1101 (Fed. Cir. 1991). Rather, § 112, second paragraph only requires the applicant to particularly point out and distinctly claim the subject matter to a degree that allows one of ordinary skill in the art would understand the scope of the claims in light of the teachings of the specification.

It is clear that the affinity ligand having no detection reagent will bind to a particular component of the sialocomplex, if present. As the specification teaches, this component may be a sialoadhesion, a glycolipid, or an antibody specifically bound to the sialocomplex. (Ex. 1, pg. 27-28). The affinity ligand with the detection reagent will bind to another component of the sialocomplex and will allow for a value of the marker to be determined or measured. (Ex. 1, pg. 27-28). As taught in Example 1, the marker being measured is human IgG with an sTn epitope (Ex. 1, pgs. 27-30). As the example illustrates, a sample is analyzed for the presence of the marker comprising human IgG and sTn epitope via a combination of affinity ligands comprising a murine anti-alpha (2,6) NeuAc antibody, which specifically binds to an epitope comprising free terminal alpha 2,6-linked sialic acid, and an anti-human IgG antibody, which specifically binds to an epitope comprising sTn. (Ex. 1, pg. 27). In this particular example, the murine anti-alpha (2,6) NeuAc antibody functions as the immobilized affinity ligand. (Ex. 1, pg. 28). Any sialocomplexes with terminal alpha 2,6-linked sialic acid become bound to the immobilized affinity ligand in the sample; the second affinity ligand comprising anti-human IgG antibody labeled with peroxidase is added, which will bind IgG antibody, which, for example, corresponds

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to step (a) of claim 10. (Ex. 1, pg. 29). The value for the marker comprising human IgG and sTn epitope that may be present in the sample can then be measured, and the measured value can be compared to a reference value, which corresponds to steps (b) and (c) of the claim 10. (Ex. 1, pg. 29 and Fig. 1). As illustrated by this example, the marker is present, if at all, in the complexes. That is, the marker's presence and value is determined by measuring the binding of both of the two affinity ligands employed in step (a) of claim 10. (App. A, pg. 1).

Reading any of claims 10, 20, 31, 46, and 54, in light of the specification, one of ordinary skill in the art would recognize the role of the second affinity ligand which does not comprise a detection reagent in the claimed method of assaying a sample for an indicator of multiple-sclerosis or a pro-multiple sclerosis immune response. For example, claim 10 calls for (a) admixing an aliquot of sample with a combination of two or more affinity ligands, wherein the two or more affinity ligands are selected from the group consisting of an anti-human antibody, an affinity ligand having binding specificity for a sialoadhesin family member, and an affinity ligand having binding specificity for an epitope comprising a terminal alpha 2,6-linked sialic acid, and wherein at least one of the affinity ligands comprises a detection reagent; (b) measuring an amount of the detection reagent, if present, which is bound to the sample in determining a value of a marker in the sample; (c) comparing the value of the marker determined to a comparative reference value. (App. 1, pg. 1).

As the claim recites, a combination of the two or more affinity ligands are selected from a group of three listed ligands. As the specification teaches, the complex being bound is a sialocomplex, defined in the specification as a complex comprising a combination of molecules which are bound to one another due to the binding specificity of at least one of the molecules; and wherein the molecules comprise a combination of two or more molecules selected from the

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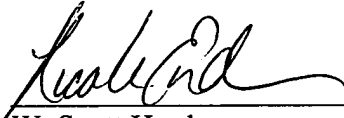
group consisting of a glycolipid having at least one terminal, alpha 2,6-linked sialic acid, a member of the sialoadhesin family and a human antibody which specifically binds an epitope comprising a terminal, alpha 2,6-linked sialic acid. (Ex. 1, pgs. 6-7). By its definition, the complex sought to be bound and measured by this method consists of at least two molecules which are bound together and exhibit a combination of the features which the combination of affinity ligands have specificity for. One of ordinary skill in the art would understand how to use the combination of at least two affinity ligands in a sample to detect a particular substance, or sialocomplex, wherein at least one of the affinity ligands comprises a detection reagent. Even without inclusion of the specific examples in the specification, scientific research principles and logic would clearly elicit the relationship of the two affinity ligand combination and ultimately apprise one of the scope of the claims.

The claims, especially in light of the teachings of the specification, clearly and unambiguously set forth the scope of the subject matter for one of ordinary skill in the art, and as such, Applicant respectfully submits that the rejection of claims 10-17, 20-28, 31-40, and 46-62 under 35 USC § 112, second paragraph is improper.

IX. CONCLUSION

For the foregoing reasons, Applicant respectfully asserts that the case is now in a condition for allowance. The Commissioner is hereby authorized to charge any additional fees, or credit any overpayment to Deposit Account No. 02-2051, referencing Attorney Docket No. 26983-50.

Respectfully submitted,

Dated: 10/9/03

By: _____
W. Scott Harders
Registration No. 42,629

N. Nicole Endejann
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10. (Original) A method for assaying a sample of an individual for an indicator of a

disease condition selected from the group consisting of MS, a pro-MS immune response, and a

combination thereof, the method comprising:

(a) admixing an aliquot of sample under biological assay conditions with a

combination of two or more affinity ligands, wherein the two or more affinity ligands are

selected form the group consisting of an anti-human antibody, an affinity ligand having binding

specificity for a sialoadhesin family member, and an affinity ligand having binding specificity

for an epitope comprising a terminal alpha 2,6-linked sialic acid, and wherein at least one of the

affinity ligands comprises a detection reagent;

(b) measuring an amount of the detection reagent, if present, which is bound to the

sample in determining a value of a marker in the sample;

(c) comparing the value of the marker determined to a comparative reference value;

wherein a difference in the value of the marker determined in the sample, when compared

to the reference value, comprises an indicator of the presence of the disease condition.

11. (Original) The method according to claim 10, wherein the sample is selected from

the group consisting of plasma, and serum.

12. (Original) The method according to claim 10, wherein at least one of the affinity

ligands comprising the detection reagent further comprises a detectable moiety.

13. (Original) The method according to claim 10, wherein at least one of the affinity ligands comprises an affinity ligand immobilized to a solid phase.

14. (Original) The method according to claim 10, wherein the anti-human antibody is selected from the group consisting of an anti-human IgG, mAb, an anti-human IgM mAb, and a combination thereof.

15. (Original) The method according to claim 10, wherein the affinity ligand having binding specificity for an epitope comprising a terminal alpha 2,6-linked sialic acid comprises an anti-sTn mAb.

16. (Original) The method according to claim 10, wherein the affinity ligand having binding specificity for a member of the sialoadhesin family comprises an affinity ligand selected from the group consisting of an anti-human MAG mAb, an anti-CD22 mAb, and a combination thereof.

17. (Original) The method according to claim 10, wherein the combination of two or more affinity ligands is a combination selected from the group consisting of anti-α (2,6) NeuAc Ab and an anti-human IgG mAb, anti-sTn mAb and anti-human IgG mAb, anti-human MAG mAb and anti-human IgM mAb, anti-human MAG mAb and anti-human IgG mAb, anti-human MAG mAb and anti-α (2,6) NeuAc Ab, anti-human MAG mAb and anti-sTn mAb, anti-human MAG mAb and anti-human CD22 mAb, anti-human CD22 mAb and anti-human IgM mAb, anti-

human CD22 mAb and anti-human IgG mAb, anti-human CD22 mAb and anti-α (2,6) NeuAc Ab, anti-human CD22 mAb and anti-sTn mAb, and a combination thereof.

20.   (Original)   A method for assaying a sample of an individual for monitoring the course of a disease condition selected from the group consisting of MS, a pro-MS immune response, and a combination thereof, the method comprising:

(a)   admixing an aliquot of sample under biological assay conditions with a combination of two or more affinity ligands, wherein the two or more affinity ligands are selected from the group consisting of an anti-human antibody, an affinity ligand having binding specificity for a sialoadhesin family member, and an affinity ligand having binding specificity for an epitope comprising a terminal alpha 2,6-linked sialic acid, and wherein at least one of the affinity ligands comprises a detection reagent;

(b)   measuring an amount of the detection reagent, if present, which is bound to the sample, in determining a value of a marker in the sample;

(c)   comparing the value of the marker determined to a comparative value selected from the group consisting of a reference value, a baseline value, and a combination thereof;

wherein a difference in the value of the marker determined in the sample, when compared to the comparative value, comprises an indicator of a change in course of the disease condition.

21.   (Original)   The method according to claim 20, wherein an indicator generated from the method may be used in a process selected from the group consisting of prognostically, for monitoring any effect of treatment on the course of the disease condition, and or for predicting a response of the disease condition to a therapeutic agent.

22.   (Original)   The method according to claim 20, wherein the sample is selected from the group consisting of plasma, and serum.

23.   (Original)   The method according to claim 20, wherein at least one of the affinity ligands comprising the detection reagent further comprises a detectable moiety.

24.   (Original)   The method according to claim 20, wherein at least one of the affinity ligands comprises an affinity ligand immobilized to a solid phase.

25.   (Original)   The method according to claim 20, wherein the anti-human antibody is selected from the group consisting of an anti-human IgG mAb, an anti-human IgM mAb, and a combination thereof.

26.   (Original)   The method according to claim 20, wherein the affinity ligand having binding specificity for an epitope comprising a terminal alpha 2,6-linked sialic acid comprises an anti-sTn mAb.

27.   (Original)   The method according to claim 20, wherein the affinity ligand having binding specificity for a member of the sialoadhesin family comprises an affinity ligand selected from the group consisting of an anti-human MAG mAb, an anti-CD22 mAb, and a combination thereof.

28. (Original) The method according to claim 20, wherein the combination of two or more affinity ligands is a combination selected from the group consisting of anti-α (2,6) NeuAc Ab and an anti-human IgG mAb, anti-sTn mAb and anti-human IgG mAb, anti-human MAG mAb and anti-human IgM mAb, anti-human MAG mAb and anti-human IgG mAb, anti-human MAG mAb and anti-α (2,6) NeuAc Ab, anti-human MAG mAb and anti-sTn mAb, anti-human MAG mAb and anti-human CD22 mAb, anti-human CD22 mAb and anti-human IgM mAb, anti-human CD22 mAb and anti-human IgG mAb, anti-human CD22 mAb and anti-α (2,6) NeuAc Ab, anti-human CD22 mAb and anti-sTn mAb, and a combination thereof.

31. (Original) A method for assaying a sample of body fluid from an individual for sialocomplexes, the method comprising:

(a) admixing an aliquot of the sample under biological assay conditions with a combination of two our more affinity ligands, wherein the two or more affinity ligands are selected from the group consisting of an anti-human antibody, an affinity ligand having binding specificity for a sialoadhesin family member, and an affinity ligand having binding specificity for an epitope comprising a terminal alpha 2,6-linked sialic acid, and wherein at least one of the affinity ligands comprises a detection reagent; and

(b) and measuring an amount of the detection reagent which is bound to sialocomplexes, if present, in determining an amount of the sialocomplexes.

32. (Original) The method according to claim 31, further comprising comparing the amount of sialocomplexes determined in the sample to a comparative value for the sialocomplexes, wherein the comparative value is selected from the group consisting of a

reference value, a baseline value, and a combination thereof; wherein a difference in the amount of the sialocomplexes determined in the sample, when compared to the comparative value, comprises an indicator of a disease condition selected from the group consisting of MS, a pro-MS immune response, and a combination thereof.

33. (Original) The method according to claim 32, wherein an indicator generated from the method may be used in a process selected from the group consisting of prognostically, for monitoring any effect of treatment on the course of the of the disease condition, and or for predicting a response of the disease condition to a therapeutic agent.

34. (Original) The method according to claim 31, wherein the sample is selected from the group consisting of plasma, and serum.

35. (Original) The method according to claim 31, wherein at least one of the affinity ligands comprising the detection reagent further comprises a detectable moiety.

36. (Original) The method according to claim 31, wherein at least one of the affinity ligands comprises an affinity ligand immobilized to a solid phase.

37. (Original) The method according to claim 31, wherein the anti-human antibody is selected from the group consisting of an anti-human IgG mAb, an anti-human IgM mAb, and a combination thereof.

38.   (Original)   The method according to claim 31, wherein the affinity ligand having binding specificity for an epitope comprising a terminal alpha 2,6-linked sialic acid comprises an anti-sTn mAb.

39.   (Original)   The method according to claim 31, wherein the affinity ligand having binding specificity for a member of the sialoadhesin family comprises an affinity ligand selected from the group consisting of an anti-human MAG mAb, an anti-CD22 mAb, and a combination thereof.

40.   (Original)   The method according to claim 32, wherein the combination of two or more affinity ligands is a combination selected from the group consisting of anti-α (2,6) NeuAc Ab and an anti-human IgG mAb, anti-sTn mAb and anti-human IgG mAb, anti-human MAG mAb and anti-human IgM mAb, anti-human MAG mAb and anti-human IgG mAb, anti-human MAG mAb and anti-α (2,6) NeuAc Ab, anti-human MAG mAb and anti-sTn mAb, anti-human MAG mAb and anti-human CD22 mAb, anti-human CD22 mAb and anti-human IgM mAb, anti-human CD22 mAb and anti-human IgG mAb, anti-human CD22 mAb and anti-α (2,6) NeuAc Ab, anti-human CD22 mAb and anti-sTn mAb, and a combination thereof.

46.   (Previously Added)   A method comprising:

        (a) admixing an aliquot of sample under biological assay conditions with a combination of two or more affinity ligands, wherein the affinity ligands are selected from the group consisting of an anti-human antibody, an affinity ligand having binding specificity for a sialoadhesin family member, and an affinity ligand having binding specificity for an epitope

comprising a terminal α 2,6-linked sialic acid, and wherein at least one of the affinity ligands comprises a detection reagent;

(b) measuring an amount of the detection reagent which is bound to the sample to determine a value of a marker in the sample;

(c) comparing the value of the marker in the sample to a comparative reference value; wherein the comparing indicates the presence or absence of a disease condition.

47.   (Previously Added)   The method according to claim 46, wherein the sample is selected from the group consisting of plasma, and serum.

48.   (Previously Added)   The method according to claim 46, wherein at least one of the affinity ligands comprising the detection reagent further comprises a detectable moeity.

49.   (Previously Amended)   The method according to claim 46, wherein at least one of the affinity ligands comprises an affinity ligand immobilized to a solid phase.

50.   (Previously Added)   The method according to claim 46, wherein the anti-human antibody is selected from the group consisting of an anti-human IgG mAb, an anti-human IgM mAb, and a combination thereof.

51.   (Previously Added)   The method according to claim 46, wherein the affinity ligand having binding specificity for an epitope comprising a terminal α 2,6-linked sialic acid comprises an anti-sTn mAb.

52. (Previously Added) The method according to claim 46, wherein the affinity ligand having binding specificity for a member of the sialoadhesin family comprises an affinity ligand selected from the group consisting of an anti-human MAG mAb, an anti-CD22 mAb, and a combination thereof.

53. (Previously Added) The method according to claim 46, wherein the combination of two or more affinity ligands is a combination selected from the group consisting of anti-α(2,6) NeuAc Ab and an anti-human IgG mAb, anti-sTn mAb and anti-human IgG mAb, anti-human MAG mAb and anti-human IgM mAb, anti-human MAG mAb and anti-human IgG mAb, anti-human MAG mAb and anti-α(2,6) NeuAc Ab, anti-human MAG mAb and anti-sTn mAb, anti-human MAG mAb and anti-human CD22mAb, anti-human CD22 mAb and anti-human IgM mAb, anti-human CD22 mAb and anti-human IgG mAb, anti-human CD22 mAb and anti-α(2,6) NeuAc Ab, anti-human CD22 mAb and anti-sTn mAb, and a combination thereof.

54. (Previously Added) A method comprising:

(a) admixing an aliquot of sample under biological assay conditions with a combination of two or more affinity ligands, wherein the affinity ligands are selected from the group consisting of an anti-human antibody, an affinity ligand having binding specificity for a sialoadhesin family member, and an affinity ligand having binding specificity for an epitope comprising a terminal α 2,6-linked sialic acid, and wherein at least one of the affinity ligands comprises a detection reagent;

(b) determining a level of the detection reagent which is bound to the sample;

(c) comparing the level of the detection reagent to a comparative reference;

(d) deriving an indicator for the presence or absence of a disease condition selected form the group consisting of MS, a pro-MS immune response, and a combination thereof based on the comparing.

55.   (Previously Added)   The method according to claim 54, wherein the indicator may be used in a process selected from the group consisting of prognostically, for monitoring any effect of treatment on the course of the disease condition, and or for predicting a response of the disease condition to a therapeutic agent.

56.   (Previously Added)   The method according to claim 54, wherein the sample is selected from the group consisting of plasma, and serum.

57.   (Previously Added)   The method according to claim 54, wherein at least one of the affinity ligands comprising the detection reagent further comprises a detectable moeity.

58.   (Previously Amended)   The method according to claim 54, wherein at least one of the affinity ligands comprises an affinity ligand immobilized to a solid phase.

59.   (Previously Added)   The method according to claim 54, wherein the anti-human antibody is selected from the group consisting of an anti-human IgG mAb, an anti-human IgM mAb, and a combination thereof.

60.   (Previously Added)   The method according to claim 54, wherein the affinity ligand having binding specificity for an epitope comprising a terminal α 2,6-linked sialic acid comprises an anti-sTn mAb.

61.   (Previously Added)   The method according to claim 54, wherein the affinity ligand having binding specificity for a member of the sialoadhesin family comprises an affinity ligand selected from the group consisting of an anti-human MAG mAb, an anti-CD22 mAb, and a combination thereof.

62.   (Previously Added)   The method according to claim 54, wherein the combination of two or more affinity ligands is a combination selected from the group consisting of anti-α(2,6) NeuAc Ab and an anti-human IgG mAb, anti-sTn mAb and anti-human IgG mAb, anti-human MAG mAb and anti-human IgM mAb, anti-human MAG mAb and anti-human IgG mAb, anti-human MAG mAb and anti-α(2,6) NeuAc Ab, anti-human MAG mAb and anti-sTn mAb, anti-human MAG mAb and anti-human CD22mAb, anti-human CD22 mAb and anti-human IgM mAb, anti-human CD22 mAb and anti-human IgG mAb, anti-human CD22 mAb and anti-α(2,6) NeuAc Ab, anti-human CD22 mAb and anti-sTn mAb, and a combination thereof.

COMPOSITIONS, ASSAY KITS, AND METHODS FOR USE RELATED TO A DISEASE CONDITION COMPRISING MULTIPLE SCLEROSIS AND/OR A PRO-MS IMMUNE RESPONSE

This application is a nonprovisional application based on earlier co-pending provisional applications Serial Nos. 60/150256, 60/151999, and 60/207577, the disclosures of which are herein incorporated by reference.

FIELD OF THE INVENTION

The field of this invention is a disease condition selected from the group consisting of multiple sclerosis (including the various forms and stages of multiple sclerosis), a humoral immune response that may accompany multiple sclerosis (a "pro-MS immune response"), and a combination thereof.

BACKGROUND OF THE INVENTION

Multiple sclerosis ("MS") is a chronic inflammatory disease of the central nervous system. The characteristic pathological feature, and still used as the primary basis for diagnosis of MS, is demyelination of the myelin sheath of neurons in the central nervous system. MS affects 250,000 to 350,000 in the United States, and approximately 1 million people worldwide. Typically, MS begins as a relapsing-remitting disease (RRMS) with periodic episodes of associated symptoms (e.g. various forms of neuritis). Often RRMS eventually changes to a progressive course of disease, secondary progressive MS (SPMS), characterized by more inflammation than RRMS or primary progressive MS, and hence, more CNS tissue damage which results in more debilitating symptoms. However, in 10 to 20% of individuals, the disease

initially develops in a progressive form known as primary progressive MS (PPMS).

There is no clear understanding of the immuno-pathogenic processes associated with MS; and, to date, their lacks evidence of a unique immunologic abnormality in individuals with MS (Whitaker, 1998, *N. Engl. J. Med.* 339:339-340; Rudick et al., 1997, *N. Engl. J. Med.* 337:1604-1611). Because of the incomplete understanding of the pathogenesis of MS, therapeutic advances have been slow to emerge. The myelin sheath and oligodendrocytes are believed to be main targets of autoreactive T cells which, when activated and reach the central nervous system (CNS), are thought to secrete proinflammatory cytokines. These cytokines are believed to induce astrocytes and leukocytes (including by activating microglia and macrophages) to secrete enzymes which damage myelin, and result in inflammation, demyelination, and axonal damage in the central nervous system characteristic in MS. Thus, studies have implicated a cell-mediated immune response, involving T cells recognizing epitopes of myelin basic protein (MBP), in the pathogenesis of MS.

Currently, the characterization of disease condition related to MS (including diagnosis, staging, monitoring disease progression, monitoring treatment effects on disease activity, and the like) is imprecise. Imaging that detects what appears to be plaques in CNS tissue is typically insufficient, by itself, to give a definitive diagnosis of MS. Often, diagnosis of MS is made only after both detection of plaques and of clinically evident neuropathy. It is clear that diagnosis of MS is usually made well

after initiation of the disease process; i.e., only after detection of a sufficient number of plaques and of clinically evident neurological symptoms. Additionally, staging of MS is typically done by subjective measurements of exacerbation of symptoms, as well of other clinical manifestations. There are difficulties in diagnosis and staging because symptoms vary widely among individuals and change frequently within the individual. Thus, there is the need for tests which can aid in the diagnosis and staging of MS. Further, presently there are no commercially available tests to evaluate for the presence of a pro-MS immune response. There is a need for laboratory tests that distinguish individuals who are more likely to have a favorable prognosis (e.g., one or more of stable remission; limited, localized disease progression; response to anti-MS therapy that either stabilizes or reduces the rate of disease progression) from individuals who are likely to have an unfavorable prognosis (e.g., individuals having undergone anti-MS therapy but who still have indications of a pro-MS immune response, and are thus still at risk for progression of the disease process; individuals having both clinically evident MS and a pro-MS immune response). Additionally, there is a need for markers that can be used as indicators for predicting whether a particular therapeutic (e.g., drug or immunotherapeutic) can effectively reduce a pro-MS immune response. Recently, one skilled in the field of MS summarized the need: "The need for reliable markers of disease activity in multiple sclerosis (MS) to better guide basic research, diagnosis, treatment, and monitoring of therapy is well-recognized."

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Therefore, a need exists for compositions and methods which may be used to characterize a disease condition selected from the group consisting of MS, a pro-MS immune response, and a combination thereof.

SUMMARY OF THE INVENTION

It is suggested that MS may actually be different diseases of similar pathology which are lumped together. According to a primary object of the present invention, provided are markers which may be determined by analyzing a body fluid of an individual having a disease condition selected from the group consisting of MS, a pro-MS immune response, and a combination thereof; wherein the markers differ in the disease condition as compared to reference values for the respective markers established from apparently healthy individuals.

In another object, one or more markers may be an indicator for a form or forms of the disease condition; may also be used to stage the development or progression of the disease condition; and may be used to monitor treatment of the disease condition.

In another object, provided are methods for assaying a clinical sample for the one or more markers. Also provided are assay kits for use in detecting or detecting and quantitating (individually or collectively referred to hereinafter as "determining") an amount of the one or more markers in practicing the methods according to the present invention.

In another object provided are detection of sialocomplexes, and methods of removing sialocomplexes from

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a body fluid of an individual having a disease condition
selected from the group consisting of MS, a pro-MS immune
response, and a combination thereof.

The above and other objects, features, and
advantages of the present invention will be apparent in the
following Detailed Description of the Invention, when read
in conjunction with the accompanying drawings.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1 is a graph illustrating the use of a combination of
affinity ligands comprising anti-α(2,6)NeuAc Ab and anti-
human IgG to generate an indicator for a disease condition
selected from the group consisting of MS, a pro-MS immune
response, and a combination thereof.

FIG. 2A is a graph illustrating the use of a combination of
affinity ligands comprising anti-MAG mAb and anti-human IgM
to generate an indicator for a disease condition selected
from the group consisting of MS, a pro-MS immune response,
and a combination thereof.

FIG. 2B is a graph illustrating the use of a combination of
affinity ligands comprising anti-MAG mAb and anti-human IgG
to generate an indicator for a disease condition selected
from the group consisting of MS, a pro-MS immune response,
and a combination thereof.

FIG. 2C is a graph illustrating the use of a combination of
affinity ligands comprising anti-MAG mAb and anti-sTn mAb to
generate an indicator for a disease condition selected from
the group consisting of MS, a pro-MS immune response, and a
combination thereof.

FIG. **3A** is a graph illustrating the use of a combination of affinity ligands comprising anti-CD22 mAb and anti-human IgM to generate an indicator for a disease condition selected from the group consisting of MS, a pro-MS immune response, and a combination thereof.

FIG. **3B** is a graph illustrating the use of a combination of affinity ligands comprising anti-CD22 mAb and anti-human IgG to generate an indicator for a disease condition selected from the group consisting of MS, a pro-MS immune response, and a combination thereof.

FIG. **3C** is a graph illustrating the use of a combination of affinity ligands comprising anti-CD22 mAb and anti-$\alpha(2,6)$NeuAc Ab to generate an indicator for a disease condition selected from the group consisting of MS, a pro-MS immune response, and a combination thereof.

FIG. **3D** is a graph illustrating the use of a combination of affinity ligands comprising anti-sTn mAb and anti-CD22 mAb to generate an indicator for a disease condition selected from the group consisting of MS, a pro-MS immune response, and a combination thereof.

DETAILED DESCRIPTION OF THE INVENTION
Definitions

The term "sialocomplexes" is used herein, for purposes of the specification and claims, to mean one or more complexes found in the body fluid of an individual having a disease condition selected from the group consistin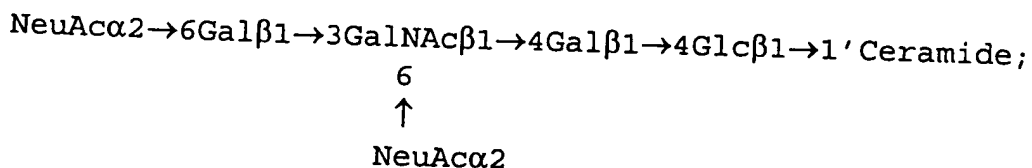g of MS, a pro-MS immune response, and a combination thereof; wherein a complex comprises a combination of molecules which are bound to one another due to the binding specificity of at

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least one of the molecules; and wherein the molecules comprise a combination of two or more molecules selected from the group of molecules consisting of a glycolipid having at least one terminal, alpha 2,6-linked sialic acid, a member of the sialoadhesin family (also know as siglec family), and a human antibody which specifically binds an epitope comprising a terminal, alpha 2,6-linked sialic acid. In a preferred embodiment, the human antibody is selected from the group consisting of IgG, IgM, and a combination thereof. The terminal, alpha 2,6-linked sialic acid comprises a terminal sialic acid alpha 2,6-linked to galactose (Gal) or a terminal sialic acid alpha 2,6-linked to N-acetyl galactosamine (GalNAc). Members of the sialo-adhesin family are known to those skilled in the art to comprise: CD22 (Siglec-2) having binding specificity for terminal, alpha 2,6-linked sialic acids (alpha 2,6-linked to either Gal or GalNAc); myelin-associated glycoprotein ("MAG", Siglec-4) having binding specificity for a terminal alpha 2,3-linked sialic acid (alpha 2,3-linked to Gal); CD33 (Siglec-3) having binding specificity for alpha 2,3-linked sialic acids, with a lesser degree of binding to alpha 2,6-linked sialic acids; sialoadhesin ("Sn"; Siglec-1) having binding specificity for terminal alpha 2,3-linked or terminal alpha 2,8-linked sialic acids; OB-BP2 (Siglec-5) having binding specificity for alpha 2,3-linked sialic acids, with a lesser degree of binding to alpha 2,6-linked sialic acids; and OB-BP1 (Siglec-6) having binding specificity for terminal, alpha 2,6-linked sialic acids (alpha 2,6-linked to GalNAc). The glycolipid comprises a ganglioside having at least one terminal alpha 2,6-linked

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sialic acid. In a preferred embodiment, the glycolipid comprises at least one terminal alpha 2,6-linked sialic acid which comprises a sialic acid selected from the group consisting of sTn antigen (a terminal sialic acid which is alpha 2,6-linked to GalNAc), a terminal sialic acid which is alpha 2,6-linked to Gal, or a combination thereof (when there is more than one terminal, alpha 2,6-linked sialic acid). The glycolipid may further comprise one or more of a terminal alpha 2,3-linked sialic acid, and a terminal alpha 2,8-linked sialic acid. Such glycolipids are known to include, but are not limited to, one or more of the alpha series of gangliosides (e.g., GD1α, GT1aα, GQ1bα, derivatives thereof which contain one or more additional terminal sialic acids alpha 2,6 linked to GalNAc, and a combination thereof). Such derivatives may comprise one or more of the following structures:

NeuAcα2→3Galβ1→3GalNAcβ1→4Galβ1→4Glcβ1→1′Ceramide;
$$\begin{matrix} 6 \\ \uparrow \\ \text{NeuAc}\alpha 2 \end{matrix}$$

NeuAcα2→3Galβ1→3GalNAcβ1→4Galβ1→4Glcβ1→1′Ceramide;
$$\begin{matrix} 6 & \uparrow \\ \uparrow & R \\ \text{NeuAc}\alpha 2 \end{matrix}$$

NeuAcα2→6Galβ1→3GalNAcβ1→4Galβ1→4Glcβ1→1′Ceramide;
$$\begin{matrix} 6 \\ \uparrow \\ \text{NeuAc}\alpha 2 \end{matrix}$$

NeuAcα2→6Galβ1→3GalNAcβ1→4Galβ1→4Glcβ1→1'Ceramide;

$$\begin{array}{ccc} & 6 & \uparrow \\ & \uparrow & R \\ & \text{NeuAcα2} & \end{array}$$
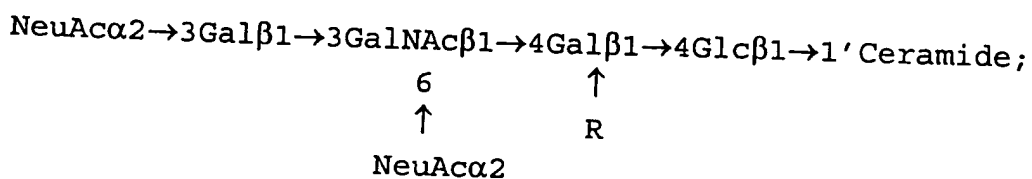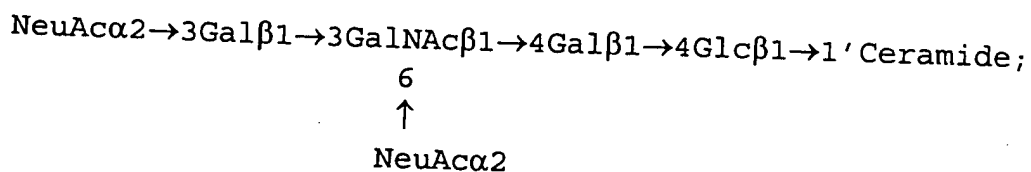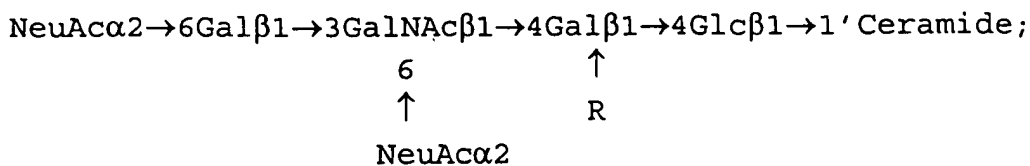
wherein R is selected from the group consisting of one
sialic acid, or two sialic acids linked together. For
example, R may comprise a 2,3-linked sialic acid or a 2,6-
linked sialic acid; R may comprise two sialic acid residues
linked together (e.g., a sialic acid which is 2,8-linked to
a sialic acid which is 2,3-linked to a Gal). In a preferred
embodiment of the present invention, the glycolipid is
produced in a greater amount and shed from CNS tissue which
is damaged from or affected by a chronic inflammatory
reaction as a result of a pro-MS immune response and/or a T
cell-mediated response, as compared to an amount produced
and shed by CNS tissue which is not undergoing a chronic
inflammatory response. Thus, in a preferred embodiment
wherein the sialocomplexes comprise a glycolipid, the
sialocomplexes further comprise one or more members of the
sialoadhesin family which specifically binds to a terminal
sialic acid of the glycolipid. In another preferred embodi-
ment, wherein the sialocomplexes comprise a glycolipid, the
sialocomplexes further comprise a human antibody which
specifically binds a terminal, alpha 2,6-linked sialic acid
of the glycolipid ("anti-α(2,6)NeuAc Ab"); and may further
comprise one or more members of the sialoadhesin family
which specifically binds to a terminal sialic acid of the
glycolipid. More preferably, the anti-α(2,6)NeuAc Ab
comprises an anti-sTn Ab. For example, wherein the sialo-
complexes comprise a terminal sTn available for binding, the

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one or more molecules complexed to sTn may be selected from the group consisting of anti-sTn Ab, a member of the sialo-adhesin which binds specifically to sTn (e.g., CD22), and a combination thereof. In a preferred embodiment, sialo-complexes are comprised of glycolipid comprising at least one terminal alpha 2,6-linked sialic acid and a terminal alpha 2,3-linked sialic acid, and one or more molecules complexed to a terminal sialic acid of the glycolipid component of the sialocomplexes. The one or more molecules may be selected from the group consisting of a molecule complexed to the terminal alpha 2,6-linked sialic acid of the glycolipid (e.g., anti-α(2,6)NeuAc Ab, CD22, or a combination thereof), a molecule complexed to the terminal alpha 2,3-linked sialic acid of the glycolipid (e.g., MAG, CD33, sialoadhesin, or a combination thereof), and a combination thereof. If the glycolipid further comprises a terminal alpha 2,8-linked sialic acid, a molecule which may be complexed to the terminal alpha 2,8-linked sialic acid may be sialoadhesin. In another preferred embodiment, the sialocomplexes comprise immune complexes comprised of the glycolipid, and human anti-sTn Ab. In another preferred embodiment, the sialocomplexes comprise glycolipid, human anti-sTn Ab, and at least one member of the sialoadhesin family selected from the group consisting of CD22, MAG, CD33, sialoadhesin, and a combination thereof. It will be apparent to one skilled in the art that sialocomplexes may comprise a single population of complexed molecules, or may comprise a mixed (regarding components of the complexes) population of complexed molecules. As an illustrative, non-limiting example, the sialocomplexes may comprise a single

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population of immune complexes comprised of glycolipid complexed to human anti-sTn Ab. In another illustrative, non-limiting example, the sialocomplexes may comprise a mixed population of sialocomplexes with each population differing in their composition of components; e.g., sialocomplexes comprised of glycolipid complexed to MAG and anti-α(2,6)NeuAc Ab, and sialocomplexes comprised of glycolipid complexed to CD22 and anti-α(2,6)NeuAc Ab.

The term "affinity ligand" is used herein, for purposes of the specification and claims, to mean a molecule which has binding specificity and avidity for a component of sialocomplexes or of the sample being analyzed, wherein the component is selected from the group consisting of a member of the sialoadhesin family, a glycolipid, and an antibody which is specifically bound to the sialocomplex (e.g., anti-α(2,6)NeuAc Ab). In a preferred embodiment, the affinity ligand may be selected from the group consisting of a monoclonal antibody having binding specificity for a sialo-adhesin family member (for binding a component comprising a sialoadhesin family member, anti-α(2,6)NeuAc Ab (for bind-ing epitope on a component wherein the epitope comprises a free terminal alpha 2,6-linked sialic acid), anti-human antibody (e.g., anti-human IgG or anti-human IgM, for binding a component comprising human anti-α(2,6)NeuAc Ab), and a combination thereof (e.g., a combination of affinity ligands, each having binding specificity for a component). For instance, where sialocomplexes comprise a glycolipid complexed to MAG, the affinity ligand may be selected from the group consisting of anti-MAG monoclonal antibody (mAb), anti-sTn mAb, and a combination thereof. In another

preferred embodiment, where sialocomplexes comprise glycolipid complexed to antibody having binding specificity for the glycolipid, the affinity ligand may be selected from the group consisting of anti-sTn Ab (for binding free sTn epitope of glycolipid in the sialo-complexes), anti-human antibody (e.g., anti-human IgG or IgM) (for binding the antibody component of the sialocom-plexes), and a combination thereof. A preferred affinity ligand may be used to the exclusion of affinity ligands other than the preferred affinity ligand.

It will be apparent to one skilled in the art that the nature of the affinity ligand may depend on the component of the sialocomplexes for which it has binding specificity. As known to those skilled in the art, affinity ligands may include, but are not limited to, lectins, antibodies (including immunoreactive fragments produced or derivatives derived from antibodies), peptides, and aptamers. Commercially available lectins that may have binding specificity for a terminal sialic acid may include, but are not limited to, *Sambucus nigra* lectin (e.g., for alpha 2,6-linked), sialic acid-binding lectin of *Helicobacter pylori* (e.g., for alpha 2,6-linked) and *Maackia Amurensis* agglutinin (e.g., for alpha 2,3-linked). Commercially available monoclonal anti-bodies that may have binding specificity for the glycolipid may comprise anti-sTn Abs including, but not limited to, mAb B72.3, and mAb HBSTn; or monoclonal antibodies having bind-ing specificity for an epitope comprising a terminal sialic acid which is alpha 2,6-linked to galactose. Monoclonal antibodies may comprise immunoreactive fragments produced or derivatives derived from an antibody molecule, which frag-

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ments retain binding function so as to be able to bind the antigen sufficiently for use in immunoassays. Such fragments are known to those skilled in the art to include $F(ab')_2$, Fab', Fab, Fv, scFV, Fd' and Fd fragments. Methods for producing the various fragments from MAbs are well known in the art (see, e.g., Plückthum, 1992, *Immunol. Rev.* 130: 152-188). For example, $F(ab')_2$ can be produced by pepsin digestion of the monoclonal antibody, and Fab' may be produced by reducing the disulfide bridges of $F(ab')_2$ fragments. Fab fragments can be produced by papain digestion of the monoclonal antibody, whereas Fv can be prepared according to methods described in U.S. Patent No. 4,642,334. Single chain antibodies can be produced as described in U.S. Patent No. 4,946,778. Aptamers can be made using methods described in U.S. Patent No. 5,789,157 (herein incorporated by reference). An affinity ligand may further comprise a detectable moiety which has been coupled to the affinity ligand using covalent or noncovalent or other means known in the art. The term "detectable moiety" is used herein, for purposes of the specification and claims, to mean a label molecule that is directly (e.g., signal-generating) or indirectly detectable. For example, detection and quantitation of the detectable moiety (as part of the affinity ligand, and when the affinity ligand is specifically bound to sialocomplexes according to the present invention) may be correlative of an amount of sialocomplexes present in a sample being analyzed. Detectable moieties may include, but are not limited to, one or more of dyes, enzymes (e.g., peroxidase, alkaline phosphatase, etc.), radioisotopes, haptens (e.g., biotin, avidin, etc.), chromophores,

luminescent molecules, fluorescent molecules, fluorescent nanocrystals, as known to those skilled in the art of diagnostics. When the detectable moiety comprises an enzyme, various substrates may be employed to provide a detectable signal such as color, light absorption, fluorescence, chemiluminescence, or the like. The particular detectable moiety is not critical to the invention, and is primarily a matter of choice and sensitivity; and may further be dependent upon the immunoassay format utilized or detection system utilized. Where an affinity ligand is not labeled with a detectable moiety, a secondary affinity ligand having a signal-generating detectable moiety may be used to specifically bind the affinity ligand (e.g., a combination of primary antibody, and labeled secondary antibody), as known to those skilled in the art.

The term "sample" is used herein, for purposes of the specification and claims, to mean a body fluid in which sialocomplexes may be found, or which may be analyzed for the markers according to the present invention. Such body fluids may comprise blood, urine, lymph, cerebrospinal fluid (CSF), or an effusion associated with multiple sclerosis. The term "sample" also encompasses a preparation which is derived from the body fluid (e.g., plasma or serum are each derived from blood).

The term "immunoassay" is used herein, for purposes of the specification and claims, to mean an assay format that may be used to determine an amount of sialo-complexes according to the present invention in a sample, or in which a combination of affinity ligands may be used in the analysis of a sample to generate one or markers which

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may comprise an indicator of a disease condition selected from the group consisting of MS, a pro-MS immune response, and a combination thereof. The immunoassay may be qualitative (detecting the presence or absence) or quantitative (detection in a manner that allows quantitation of the amount); e.g., in determining an amount of sialocomplexes according to the present invention. As appreciated by those skilled in the art, various formats of immunoassays may be used in the analysis of a sample. Factors which influence which format is utilized may include, but is not limited to, the affinity ligands utilized, the components being assayed for, sensitivity of the determination, and preference of the user of the format. In principle, conventional immunoassays known to those skilled in the art may have a suitable format which may be utilized in the methods according to the present invention. Such immunoassay formats are known to those skilled in the art to include, but are not limited to, an inhibition-type immunoassay, a competitive immunoassay, an interference immunoassay, an enzyme-linked immunosorbent assay (ELISA), a competitive ELISA, a sandwich immunoassay, a solid phase immunoassay, heterogeneous competitive immunoassay (liquid phase is separated from the solid phase), a kinetic immunoassay, a precipitation immunoassay, an agglutination immunoassay, an enzyme immunoassay, a fluorescence immunoassay, a radioimmunoassay, a liquid phase immunoassay, and other immunoassays.

The terms "differs" and "difference" is used herein relative to a comparison between one or more markers or amounts determined from a sample and that of a comparative value (reference value or baseline value), for purposes

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of the specification and claims, to mean that the markers (e.g., amount of sialocomplexes, or pattern of immunoreactivity when the sample is analyzed) determined in a sample falls outside the comparative value (e.g., outside a range of normal clinical values for the indicator that is established by clinical studies of apparently healthy individuals, the range of normal clinical values being referred to as a "reference value"). Hence, where a marker determined according to the present invention differs with respect to a reference value, the marker may comprise an indicator for a disease condition selected from the group consisting of MS, a pro-MS immune response, and a combination thereof. In a preferred use, the term "differs" is used herein, for purposes of the specification and claims, to mean that there is a statistically significant difference between a marker determined according to the present invention from a clinical sample, and a comparative value for the marker selected from the group consisting of a reference value (comprising a predetermined value or a range of values in the same type of body fluid of apparently healthy individuals), a baseline value (comprising a predetermined value from one or more previous samples of the same type of body fluid obtained from the same individual being tested), and a combination thereof. In a preferred embodiment, a statistically significant difference between a first value and a second value in a comparison may comprise the first value being at least about two standard deviations outside the mean of the second value. In general, the methods, assay formats, and compositions, of the present invention are used to generate indicators for a disease

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condition selected from the group consisting of MS, a pro-MS immune response, and a combination thereof; in providing an additional parameter to a competent health professional in making a medical opinion.

The term "pro-MS immune response" is used herein, for purposes of the specification and claims, to mean a humoral immune response induced against an epitope comprising a terminal alpha 2,6-linked sialic acid (e.g., comprising sialyl Tn or sTn which comprises a terminal sialic acid alpha 2,6-linked to GalNAc) of a shed antigen (glyco-molecule), resulting in production of IgG antibody against the epitope ("anti-α(2,6)NeuAc Ab"), and complexes comprised of the shed antigen comprising the epitope complexed to anti-α(2,6)NeuAc Ab, and may further comprise one or more members of the sialoadhesin family; wherein the shed antigen is released or produced particularly in relation to CNS tissue damage characteristic of MS during the MS disease process. In a preferred embodiment, the resultant complexes bind to and induce Fc receptor-expressing cells (e.g., one or more cell types selected from the group consisting of granulocytes, macrophages, microglia, activated mast cells, astrocytes, oligodendrocytes) which results in the release of inflammatory mediators (e.g., cytokines and/or tissue degradative enzymes) which may promote (contribute to) CNS tissue damage characteristic of MS (e.g., demyelination and plaques characteristic of MS). A similar immune response, a pro-tumor immune response, has been described in co-pending U.S. application Serial No. 09/435,289 (the disclosure of which is herein incorporated by reference). In a preferred embodiment, the anti-α(2,6) NeuAc Ab is induced by a shed

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antigen comprising glycolipid, as previously described herein in more detail. Cellular markers for a pro-MS immune response have been described in detail in co-pending U.S. application Serial No. 60/150256, the disclosure of which is herein incorporated by reference.

The term "biological assay conditions" is used herein, for purposes of the specification and claims, to mean those conditions under which an affinity ligand can specifically bind to the molecule for which it has binding specificity (e.g., a component of the sialocomplexes according to the present invention). As known to those skilled in the art, such conditions may include one or more of: a pH range of from about 5 to about 9, ionic strengths such as that ranging from distilled water to about 1 molar sodium chloride, and a temperature in the range of from about 4°C to about 45°C; and may further include a time sufficient for binding to occur (e.g., in a range of from about 10 minutes to about 2 hours).

There is a need for clinical tests which can aid in the diagnosis of MS. Additionally, there is a need for clinical tests which can detect one or more of: individuals at risk of developing MS, initiation of inflammatory processes preceding development of a pattern of clinical symptoms characteristic of MS, detection of MS at an earlier point in time than currently available techniques, and monitoring the progression of MS (e.g., from RRMS to SPMS). Further, presently there are no commercially available tests to evaluate for the presence of a pro-MS immune response. There is a need for laboratory tests that distinguish

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individuals who are more likely to have a favorable prognosis (e.g., one or more of stable remission; limited, localized disease progression; response to anti-MS therapy that either stabilizes or reduces the rate of disease progression) from individuals who are likely to have an unfavorable prognosis (e.g., individuals having undergone anti-MS therapy but who still have indications of a pro-MS immune response, and are at risk for progression; individuals having both clinically evident MS and a pro-MS immune response). Additionally, there is a need indicators for predicting whether a particular therapeutic (e.g., drug or immunotherapeutic) can effectively reduce a disease process comprising MS and/or a pro-MS immune response.

The present invention provides a method for analyzing a sample from an individual being screened for, or suspected of having, or known to have a disease condition selected from the group consisting of MS, a pro-MS immune response, and a combination thereof. The method of the present invention is achieved because of a discovery that a combination of affinity ligands can be used in an immuno-assay to generate one or more indicators that appear to differ in individuals having a disease condition selected from the group consisting of MS, a pro-MS immune response, and a combination thereof, as compared to a comparative values determined from healthy controls or in individuals having inflammatory diseases other than MS. As will be apparent from the following descriptions, the methods and assay kits according to the present invention may be used to confirm a suspicion of MS (based on either initial symptoms or other clinical evidence initially presented), to deter-

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mine the state of the disease condition in the individual, to monitor the course of the disease condition (e.g., progression; or regression after treatment), and the like. A marker determined according to the present invention may be used individually or in a panel of markers for detecting a disease condition; i.e., to distinguish between primary progressive MS, relaxing remitting MS, and secondary progressive MS, and to detect the presence or absence of a pro-MS immune response. For example, to determine the disease condition, the observed values for the markers determined from analysis of the clinical sample are compared to reference values, wherein the markers comprise indicators which are correlated to a disease condition or form thereof. A marker determined according to the present invention may be used individually or in a panel of markers for staging the disease condition; i.e., to distinguish between relaxing remitting MS, and secondary progressive MS. For example, to determine the stage of the disease condition, the observed values for the markers determined from analysis of the clinical sample are compared to reference values, wherein the markers comprise indicators which are correlated to a stage of a disease condition. A marker determined according to the present invention may be used individually or in a panel of markers for staging the disease condition; i.e., to distinguish between relaxing remitting MS, and secondary progressive MS. For example, to determine the stage of the disease condition, the observed values for the markers determined from analysis of the clinical sample are compared to comparative values, wherein when the observed values of the markers differ from the respective comparative values,

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the markers comprise indicators which are correlated to a stage of a disease condition. A marker determined according to the present invention may be used individually or in a panel of markers for monitoring the course of the disease condition; i.e., whether the disease condition is progressing (e.g., worsening, and therefore predictive of a worsening in symptoms or severity) in course, or has stabilized, or has improved by regression (e.g., a change in the one or markers to within or closer to the range of the comparative values). For example, to determine the course of the disease condition, the observed values for the markers determined from analysis of the clinical sample are compared to comparative values, wherein when the observed values of the markers differ from the respective comparative values, the markers comprise indicators which are correlated to the course of a disease condition.

Assay kits are provided for performing the above described methods. In one embodiment, the assay kit comprises a combination of affinity ligands comprising an anti-human Ab; at least one affinity ligand having binding specificity for a member of the sialoadhesin family; and an affinity ligand having binding specificity for an epitope comprising a terminal alpha 2,6-linked sialic acid. The sample may be assayed using a combination of the affinity ligands in determining one or more markers or an amount of sialocomplexes according to the present invention. One or more affinity ligands of the combination of affinity ligands may further comprise a detectable moiety (in comprising a "detection reagent"). In a preferred embodiment, the anti-human antibody is selected from the group consisting of an

anti-human IgG mAb, an anti-human IgM mAb, and a combination thereof. In a preferred embodiment, the affinity ligand having binding specificity for an epitope comprising a terminal alpha 2,6-linked sialic acid comprises an anti-sTn mAb. In another embodiment, the assay kits may include various components, depending on the complexity of the screening method utilized for determining an amount of sialocomplexes according to the present invention. Assay kits may further comprise one or more reagents comprising a reference standard comprising a known amount of the one or more markers (e.g., the sialocomplexes) desired to be detected in the assay, instructions for use of the assay kit and components, and optionally, other accessories useful in carrying out the methods of the present invention.

A method according to the present invention may be performed in a liquid phase or a solid phase of an immuno-assay. In a preferred immunoassay format for assaying a clinical sample in a method according to the present invention, the immunoassay comprises: affinity ligand, having binding specificity for a component of the sialo-complexes, which is immobilized to a support surface in forming a solid phase ("immobilized affinity ligand"); contacting and reacting the solid phase with a sample under biological assay condition sufficient for sialocomplexes that may be present in the sample to be specifically bound by the immobilized affinity ligand; and then determining an amount of sialocomplexes bound to the immobilized affinity ligand. In a preferred embodiment, determining an amount of sialocomplexes bound to the immobilized affinity ligand may be accomplished by contacting and reacting the solid phase

containing the sample under biological assay conditions with one or more affinity ligands having binding specificity for the sialocomplexes (e.g., components thereof) for detecting the sialocomplexes (the affinity ligand comprising a "detection reagent"), and then measuring the amount of the detection reagent which remains bound to the solid phase (e.g., by measuring the amount of signal generated by the detectable moiety portion of the detection reagent), wherein the amount of the bound detection reagent is measured and correlated to the amount of sialocomplexes in the sample.

In this preferred embodiment, the affinity ligand is immobilized onto a solid phase. As known to those skilled in the art, a solid phase may comprise a solid support that may include, but is not limited to, a bead (e.g., polystyrene, paramagnetic, etc.), test tube, reaction vessel other than a test tube, reaction chamber, microtiter plate well, dipstick, slot for performing reactions therein (e.g., of a microarray), a sheet for performing reactions thereon (e.g., nitrocellulose or derivatized sheet material), slides, films, membranes, or the like. The particular solid phase is not critical, and any solid phase suitable for an immunoassay may be used; e.g., where non-specific binding may be minimized, and where the solid phase is suitable for use in a convenient protocol without significantly interfering with measurement of an amount of an analyte such as sialocomplexes. The affinity ligand may be immobilized to the solid phase using covalent or noncovalent means as known to those skilled in the art. Such means will depend on factors including the chemical nature of the affinity ligand, and the nature of surface of the solid support used in forming

the solid phase. The particular manner in which the affinity molecule is immobilized is not critical so long as it is compatible with the intended purpose of an immuno-assay, and with the methods according to the present invention and the reagents used therein. For example, where the affinity ligand is a protein such as a lectin or antibody, the affinity ligand is adsorbed onto a solid surface in forming a solid phase. Techniques for coating affinity ligands onto solid surfaces are well known in the art. Typically, after immobilizing the affinity ligand, the solid phase is then treated to reduce non-specific binding to any subsequent reactants added to the solid phase. More particularly, non-specific binding sites on the solid phase (i.e., those sites not occupied by affinity ligand) are generally blocked using blocking agents alone, or in con-junction with a detergent (the latter reduces non-specific binding). Preferred blocking agents are known to those skilled in the art to include, but are not limited to, milk, bovine serum albumin, casein, gelatin, and betaglobin. Detergents include non-interfering concentrations of Tween, NP40, and the like.

After preparing the solid phase, the sample (e.g., portion or aliquot of the sample) may be added to and contacted with the solid phase and reacted under biological assay conditions sufficient for sialocomplexes, if present in the sample, to be bound by the immobilized affinity ligand of the solid phase. It may be desirable to add to separately assayable solid phases one or more reference standards comprising a known amount of the sialocomplexes to be measured so as to aid in interpreting the results of the

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assay (e.g., to ensure that the affinity ligands used still maintain their binding ability). After such incubation, the solid phase may be washed so as to remove from the solid phase any unbound or non-specifically bound contents of the sample. Generally, one to several washes with a solution compatible with the immunoassay will be sufficient to wash the unbound or non-specifically bound contents from the solid phase. After washing, a solution containing the detection reagent may be added to and contacted with the solid phase. Generally, the detection reagent should be added in a concentration that is sufficient to saturate all or substantially all sialocomplexes that may be bound to immobilized affinity ligand in the solid phase; and reacted under biological assay conditions sufficient for the detection reagent to contact and bind sialocomplexes that may be present in the solid phase. Typically, after incubation, the solid phase is washed to remove any unbound or non-specifically bound detection reagent. After the wash, detectable signal, generated from the detectable moiety of the detection reagent, that may be bound sialocomplexes in the solid phase, is detected by means conventional for the immunoassay format being utilized. Measurement of bound detection reagent may be used to quantitatively or qualitatively determine an amount of sialocomplexes in the sample. For quantitative analysis, units of measurement may be computed, such as through use of an absorbance value calculated for the sample, as compared to absorbance values for reference standards that are run in parallel and as plotted on a standard curve. Depending on the type of detection reagent (affinity ligand and

detectable moiety) used, and immunoassay format utilized, other units may be used to generate a measurement, such as optical density, or amount of fluorescent emission. Thus, an amount of sialocomplexes determined using an immunoassay may be expressed as a relative value (e.g., relative to means of measurement such as absorbance), or expressed as an actual value (e.g., in weight per volume). Accordingly, in a preferred method according to the present invention, the immunoassay comprises: a combination of affinity ligands comprising two affinity ligands selected from the group consisting of an anti-human Ab, an affinity ligand having binding specificity for a member of the sialoadhesin family, and an affinity ligand having binding specificity for an epitope comprising a terminal alpha 2,6-linked sialic acid; and wherein at least one of the affinity ligands comprises an immobilized affinity ligand, and at least one of the affinity ligands comprises a detection reagent for use in analyzing the sample in the solid phase.

In another preferred embodiment, provided is a method for analyzing a sample for markers associated with a a disease condition selected from the group consisting of MS, a pro-MS immune response, and a combination thereof. The method may use an immunoassay format essentially as described above. The clinical sample is added to and incubated with the immobilized affinity ligand under biological assay conditions. The one or more affinity ligands comprising the detection reagent is then added to the solid phase. Detection and measurement of the bound detection reagent in the solid phase containing the sample being analyzed results in a value that comprises a marker

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which may be compared to a comparative value, wherein where
the value of the marker differs as compared to the compara-
tive value, the marker comprises an indicator which may be
correlated to a disease condition selected from the group
consisting of MS, a pro-MS immune response, and a combina-
tion thereof.   -

EXAMPLE 1

This example illustrates use of a combination of
affinity ligands to generate an indicator from analysis of a
sample. Also, this example illustrates one embodiment of
the present invention wherein an amount of sialocomplexes is
determined in a sample by an immunoassay according to the
present invention. In this illustrative example, the sample
was analyzed by contact under biological assay conditions
with a combination of affinity ligands comprising a murine
anti-α(2,6)NeuAc Ab for binding specifically to an epitope
comprising free (unbound) terminal alpha 2,6-linked sialic
acid, and an anti-human IgG antibody for binding specific-
ally to a human antibody. In a preferred embodiment, the
human antibody component being assayed for is an antibody
having binding specificity for an epitope comprising sTn;
and hence, determination of an amount of human antibody in
the sample which differs from a reference value is an
indicator of a disease condition selected from the group
consisting of MS, a pro-MS immune response, and a combina-
tion thereof. To illustrate this embodiment, wells of a
polystyrene microtiter plate (a solid phase) were coated
with the murine anti-α(2,6)NeuAc Ab comprising anti-sTn mAb
as the immobilized affinity ligand by adding 100 μl/well of

a 10 µg/ml solution diluted in phosphate buffered saline (PBS). The plate was incubated overnight at 4°C. The solution was then removed, and the wells were washed to remove any unbound mAb. The plates were then blocked with a blocking solution comprising 1% bovine serum albumin, 0.05% Tween, and 2% goat serum for 1 hour at room temperature, and then washed to remove excess blocking solution from the solid phase.

The samples reacted with the solid phase under biological assay conditions comprised plasma from: individuals with no apparent disease or pathology ("Reference Value"); individuals having relapsing-remitting (RRMS), or secondary progressive (SPMS), or primary progressive (PPMS) form of multiple sclerosis. The plasma was diluted with 1:50 with buffer (PBS + 0.05% Tween) before addition to the solid phase. Aliquots of 100 µl were added to appropriately designated wells of the microtiter plate. The plate was then incubated for 1 hour at room temperature, and then washed with buffer to remove unbound or non-specifically bound plasma components from the solid phase. The affinity ligand comprising the detection reagent for assaying the sample comprised anti-human IgG labeled with peroxidase (e.g., horseradish peroxidase) for detecting antibody of the IgG class, if present, as a component of the sample bound to the solid phase. The detection reagent comprising anti-human IgG labeled with peroxidase was added to the appropriately designated wells at the dilution recommended by the manufacturer. The plate was then incubated for 1 hour at room temperature, and then washed with buffer to remove unbound or non-specifically bound, labled anti-human IgG from the

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solid phase. Substrate, comprising tetramethyl benzidine (TMB) was then added to determine peroxidase activity of bound etection reagent, if present. Resultant color development was quantitated at 450 nanometers using a plate-reading spectrophotometer. FIG. 1 illustrates an amount selected from the group consisting of a marker comprising an observed level of immunoreactivity determined from the samples, and an amount of sialocomplexes determined from the samples, as measured by absorbance at 450 nm (with a correction at absorbance 570) and expressed as the mean absorbance.

As illustrated in FIG. 1, the observed value for a marker comprising human IgG and sTn epitope that may be present together in clinical samples from individuals with RRMS or with SPMS differs (is increased) as compared to the reference value (FIG. 1 "Control"). However, such an increase in this marker was not observed in clinical samples from an individual having a disease condition comprising PPMS, a slow progressing form of MS. These results suggest that (a) a pro-MS immune response, as identified by an increase (as compared to a reference value) in this marker, and which may represent sialocomplexes comprising IgG containing-immune complexes, may be present in individuals with RRMS, or with SPMS; (b) that this marker comprises an indicator that may be used to distinguish different forms of MS (e.g., PPMS lacks the indicator, and hence, may be distinguished from RRMS and SPMS); and (c) that a combination of affinity ligands comprising an affinity ligand having binding specificity for an epitope comprising sTn, and anti-human Ab (anti-human IgG), may be used to generate

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an indicator of a disease process selected from the group consisting of RRMS, SPMS, a pro-MS immune response, or a combination thereof (i.e., RRMS and a pro-MS immune response, or SPMS and a pro-MS immune response).

EXAMPLE 2

This example illustrates another embodiment of using a combination of affinity ligands to generate an indicator from analysis of a sample. Also illustrated is other embodiments in which an amount of sialocomplexes is determined in a sample by an immunoassay according to the present invention. In this illustrative embodiment, a murine anti-human MAG antibody (mAb) was used as the immobilized affinity ligand for binding a component comprising myelin-associated glycoprotein (MAG) in the sample being analyzed. An affinity ligand comprising a detection reagent comprised anti-human antibody, for binding a human antibody component of the sample; or alternatively, comprised an anti-α(2,6) NeuAc Ab, for binding a component of the sample comprising a free, terminal alpha 2,6-linked sialic acid. In this illustration, the anti-human antibody comprised either anti-human IgG or anti-human IgM; and the anti-α(2,6)NeuAc Ab comprised anti-sTn mAb. For this illustration, the methodology for the immunoassay was essentially performed as described in Example 1 herein. As illustrated in FIG. 2A, the immunoassay for determining human IgM and MAG showed that the amount of this marker (e.g., comprising sialocomplexes) may be present in amount that differs (an increase) in clinical samples from individuals having a disease condition selected from the group

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consisting of RRMS, SPMS, and PPMS, as compared to the

reference value (FIG. 2A, "Control"). As illustrated in

FIG. 2B, the immunoassay for determining human IgG and MAG

showed that the amount of this marker (e.g., comprising

sialocomplexes) may be present in amount that differs (an

increase) in clinical samples from individuals having a

disease condition selected from the group consisting of

RRMS, and SPMS, as compared to the reference value (FIG. 2B,

"Control"). However, such an increase in amount of this

marker (comprising human IgG and MAG) was not observed in

samples from an individual having a disease condition

comprising PPMS, a slow progressing form of MS. Similarly,

as illustrated in FIG. 2C, the immunoassay for MAG and an

epitope comprising free terminal alpha 2,6-linked sialic

acid showed that the amount of this marker (e.g., comprising

sialocomplexes) may be present in amount that differs (an

increase) in clinical samples from individuals having a

disease condition selected from the group consisting of

RRMS, and SPMS, as compared to the reference value (FIG. 2C,

"Control"). However, such an increase in an amount of this

marker (comprising MAG and free terminal alpha 2,6-linked

sialic acid) was not observed in samples from an individual

having a disease condition comprising PPMS, a slow progress-

ing form of MS. The results from FIGs. 2A-2C further

suggest that (a) a pro-MS immune response, as identified by

an increase (as compared to a reference value) in these

markers (e.g., such as sialocomplexes) comprising IgG

containing-complexes, may be present in individuals having a

disease condition selected from the group consisting of

RRMS, SPMS, and a combination thereof; (b) that these

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markers comprise indicators that may be used to stage and distinguish different forms of MS (e.g., PPMS lacks the indicator comprising IgG-containing complexes, and hence, may be distinguished from RRMS and SPMS; SPMS have a higher observed value of this marker than RRMS, a form of MS that typically precedes SPMS); and (c) that a combination of affinity ligands comprising an affinity ligand having binding specificity for MAG in combination with any one of anti-human IgG or anti-$\alpha(2,6)$NeuAc Ab may be used to generate an indicator of a disease condition selected from the group consisting of RRMS, SPMS, a pro-MS immune response, or a combination thereof (i.e., RRMS and a pro-MS immune response, or SPMS and a pro-MS immune response).

EXAMPLE 3

This example illustrates another embodiment of using a combination of affinity ligands to generate an indicator from analysis of a sample. Also illustrated are other embodiments wherein an amount of sialocomplexes is determined in a sample by an immunoassay according to the present invention. In this illustrative embodiment, murine anti-human CD22 antibody was used as an immobilized affinity ligand for binding a CD22 component of the sample. The affinity ligand comprising the detection reagent comprised anti-human antibody, for binding a human antibody component of the sample. Alternatively, anti-$\alpha(2,6)$NeuAc Ab was used as an immobilized affinity ligand for binding free, terminal alpha 2,6-linked sialic acids of the sample; and the affinity ligand comprising the detection reagent comprised anti-human CD22 antibody (mAb). In this illustration, the

anti-human antibody comprised either anti-human IgG or anti-human IgM; and the anti-α(2,6)NeuAc Ab comprised anti-sTn mAb. For this illustration, the methodology for the immunoassay was essentially performed as described in Example 1 herein. As illustrated in FIG. 3A, the immunoassay for human IgM and CD22 showed that the amount of this marker (e.g., an amount of sialocomplexes) may be present in amount that differs (an increase) in clinical samples from individuals having a disease condition selected from the group consisting of RRMS, and PPMS, as compared to the amount of the reference value (FIG. 3A, "Control"). As illustrated in FIG. 3B, the immunoassay for human IgG and CD22 showed that the amount of this marker (e.g., an amount of sialocomplexes) may be present in amount that differs (an increase) in clinical samples from individuals having a disease condition selected from the group consisting of RRMS, and SPMS, as compared to the reference value (FIG. 3B, "Control"). However, such an increase in this marker (comprising human IgG and CD22) was not observed in samples from an individual having a disease condition comprising PPMS, a slow progressing form of MS. As illustrated in FIGs. 3C (anti-sTn mAb as the immobilized affinity ligand and anti-CD22 mAb as the detection reagent) and 3D (anti-CD22 mAb as the immobilized affinity ligand and anti-sTn mAb as the detection reagent): a) the marker comprising CD22 and free terminal alpha 2,6-linked sialic acid showed that the amount of this marker (e.g., amount of sialocomplexes) may be present in amount that differs (an increase) in clinical samples from an individual having a disease condition comprising PPMS, as compared to the reference value (FIG.

3C, "Control"). A similar pattern to that illustrated in FIGs. 3C & 3D was seen when anti-MAG mAb was used as the immobilized affinity ligand, and anti-CD22 was used as the detection reagent. However, such a significant increase in this marker (comprising CD22 and free terminal alpha 2,6-linked sialic acid; or comprising MAG and CD22) was not observed in clinical samples from individuals having a disease condition selected from the group consisting of SPMS, and RRMS. The results from FIGs. 3A-3D further suggest that (a) a pro-MS immune response, as identified by an increase (as compared to a reference value) in these markers (e.g., sialocomplexes) comprising IgG containing-immune complexes, may be present in individuals having a disease condition selected from the group consisting of RRMS, and SPMS; (b) that different forms or stages of MS may be distinguishable by these markers individually or as a panel of markers; and (c) that a combination of affinity ligands comprising an affinity ligand having binding specificity for CD22, and anti-human IgG may be used to generate an indicator of a disease condition selected from the group consisting of RRMS, SPMS, a pro-MS immune response, and a combination thereof (i.e., RRMS and a pro-MS immune response, or SPMS and a pro-MS immune response). Thus, an indicator of the presence of a disease condition selected from the group consisting of MS, a pro-MS immune response, and a combination thereof, may comprise an indicator comprising an amount of IgG-containing complexes which differs (e.g., significantly increased), as compared to the reference value.

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EXAMPLE 4

In this embodiment, provided are various illus-
trations of the methods according to the present invention.
As illustrated in Examples 1-3 herein, preferred combina-
tions of two affinity ligands which may be used in these
methods include one or more of: anti-α(2,6)NeuAc Ab and
anti-human IgG mAb; anti-sTn mAb and anti-human IgG mAb;
anti-human MAG mAb and anti-human IgM mAb; anti-human MAG
mAb and anti-human IgG mAb; anti-human MAG mAb and anti-
α(2,6)NeuAc Ab; anti-human MAG mAb and anti-sTn mAb; anti-
human MAG mAb and anti-human CD22 mAb; anti-human CD22 mAb
and anti-human IgM mAb; anti-human CD22 mAb and anti-human
IgG mAb; anti-human CD22 mAb and anti-α(2,6) NeuAc Ab;
anti-human CD22 mAb and anti-sTn mAb; and anti-sTn mAb and
anti-human CD22 mAb. Thus, an indicator is generated by use
in an immunoassay of a combination of affinity ligands
according to the present invention, or by use in an immuno-
assay of more than one combination of affinity ligands.
Preferred combinations of affinity ligands may be used to
generate individual indicators or a panel of indicators
which may be used in diagnostics or prognostics. For
example, and as shown in either FIG. 3C or FIG. 3D, an
indicator generated using a combination of affinity ligands
comprising an affinity ligand which binds specifically to
CD22, and an affinity ligand which binds specifically to a
terminal alpha 2,6-linked sialic acid (e.g., sTn) may be
indicative of a disease condition comprising primary
progressive MS. As an illustrative example of generating a
panel of indicators, one or more indicators generated from
using any one combination of two affinity ligands or a

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plurality of combinations of two affinity ligands comprising anti-sTn mAb and anti-human IgG mAb, anti-human MAG mAb and anti-human IgM mAb, anti-human MAG mAb and anti-human IgG mAb, anti-human MAG mAb and anti-sTn mAb, and anti-human CD22 mAb and anti-human IgG mAb, may be used in a panel with an indicator generated from a combination of affinity ligands comprising anti-CD22 mAb and anti-human IgM to distinguish a disease condition comprising SPMS from a disease condition comprising RRMS.

In a preferred embodiment according to the present invention a preferred body fluid, of which a sample is to be analyzed, comprises a blood component selected from the group consisting of serum, and plasma. Accordingly, provided is a method for assaying a sample of an individual for an indicator of a disease condition selected from the group consisting of MS, a pro-MS immune response, and a combination thereof, the method comprising: (a) admixing an aliquot of sample under biological assay conditions with a combination of two or more affinity ligands, wherein the two or more affinity ligands are selected from the group consisting of an anti-human antibody, an affinity ligand having binding specificity for a sialoadhesin family member, and an affinity ligand having binding specificity for an epitope comprising a terminal alpha 2,6-linked sialic acid, and wherein at least one of the affinity ligands comprises a detection reagent; (b) measuring an amount of the detection reagent, if present, which is bound to the sample in determining a value of a marker in the sample;
(c) comparing the value of the marker determined to a comparative reference value;

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wherein a difference in the value of the marker determined in the sample, when compared to the reference value, comprises an indicator of the presence of the disease condition. As apparent to those skilled in the art, the sample analyzed, and sample from which the comparative value was determined, should comprise the same body fluid type (i.e., values of a serum marker should be compared to a reference value determined from assay of sera).

In another preferred embodiment, provided is a method for assaying a sample of an individual for monitoring the course of a disease condition selected from the group consisting of MS, a pro-MS immune response, and a combination thereof, the method comprising: (a) admixing an aliquot of sample under biological assay conditions with a combination of two or more affinity ligands, wherein the two or more affinity ligands are selected from the group consisting of an anti-human antibody, an affinity ligand having binding specificity for a sialoadhesin family member, and an affinity ligand having binding specificity for an epitope comprising a terminal alpha 2,6-linked sialic acid, and wherein at least one of the affinity ligands comprises a detection reagent; (b) measuring an amount of the detection reagent, if present, which is bound to the sample, in determining a value of a marker in the sample; (c) comparing the value of the marker determined to a comparative value selected from the group consisting of a reference value, a baseline value, and a combination thereof; wherein a difference in the value of the marker determined in the sample, when compared to the comparative value, comprises an indicator of a change in course of the

disease condition.   This method may be used in a prognostic manner (e.g., the sample analyzed and sample from which the baseline value was measured comprise samples obtained at different points of time relative to the individual); or for monitoring (during or after treatment of the individual's disease) the effect, if any, of treatment on the course of the disease condition; or for predicting whether a particular therapeutic agent (e.g., drug, immunotherapeutic, or the like) can effectively reduce or stabilize the course of the disease condition.

For example, if the individual having a disease condition comprising either SPMS and PMSIR, or RRMS and PMSIR, is undergoing treatment to reduce or suppress the pathology associated with the underlying disease condition, and such treatment is efficacious, the value of the marker (e.g., representative of the amount of sialocomplexes detected by a combination of anti-sTn mAb and anti-human IgG in the sample analyzed after treatment) may show a significant decrease as compared to the baseline value (e.g., representative of the amount of the same type of sialo-complexes from a previous determination). Such a significant decrease may comprise an indicator that the disease condition is being suppressed or reduced, which may also be an indicator of a favorable response to treatment. In another illustrative embodiment, wherein the value of the marker (e.g., representative of the amount of sialocomplexes detected by a combination of anti-MAG mAb and anti-human IgG) is significantly increased as compared to the baseline value (e.g., representative of the amount of the same type of sialocomplexes from a previous determination), such a

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significant increase may comprise an indicator that the disease condition is progressing in course (e.g., an induction of a more inflammatory disease process underlying the disease condition), which may also be an indicator of an unfavorable prognosis. Such indicators resulting from the methods according to the present invention (e.g., prognostic or diagnostic), is provided as an additional parameter to a competent health professional in making a medical opinion. As will be apparent to those skilled in the art, the comparative values may vary depending upon such factors which include, but are not limited to, the type of clinical sample analyzed (e.g., origin or type of body fluid), the nature of the one or more detection reagents used (binding specificity, detectable moiety, etc.), and the process and instrumentation used to detect the bound detection reagent according to the present invention.

In a similar embodiment of the present invention, provided is a method for assaying a sample of body fluid from an individual for sialocomplexes, the method comprising: (a) admixing an aliquot of the sample under biological assay conditions with a combination of two or more affinity ligands, wherein the two or more affinity ligands are selected from the group consisting of an anti-human antibody, an affinity ligand having binding specificity for a sialoadhesin family member, and an affinity ligand having binding specificity for an epitope comprising a terminal alpha 2,6-linked sialic acid, and wherein at least one of the affinity ligands comprises a detection reagent; and (b) measuring an amount of the detection reagent which is bound to sialocomplexes, if

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present, in determining an amount of the sialocomplexes. The method may further comprise comparing the amount of sialocomplexes determined in the sample to a comparative value for the sialocomplexes, wherein the comparative value is selected from the group consisting of a reference value, a baseline value, and a combination thereof; wherein a difference in the amount of the sialocomplexes determined in the sample, when compared to the comparative value, comprises an indicator of a disease condition selected from the group consisting of MS, a pro-MS immune response, and a combination thereof. In a more preferred embodiment, the sample analyzed comprises serum or plasma. As previously described herein in more detail, the indicator may be used by itself, or in combination with other indicators generated from the sample in forming a panel of indicators, in a diagnostic manner (e.g., when compared to a reference value), prognostic manner (e.g., when compared to a baseline value and/or a reference value), or for monitoring the course of the disease condition (e.g., when compared to a baseline value), in providing an additional parameter to a competent health professional in making a medical opinion.

EXAMPLE 5

The present invention also provides for: (a) removing sialocomplexes from the blood of an individual having a disease process selected from the group consisting of MS, a pro-MS immune response, and a combination thereof, wherein the process for removing the sialocomplexes may be achieved by plasma exchange as known to those skilled in the art of treating autoimmune disorders such as multiple

sclerosis; and (b) isolated and purified sialocomplexes that may be isolated and purified from one or more body fluids of an individual having having a disease process selected from the group consisting of MS, a pro-MS immune response, and a combination thereof MS. It will be apparent to one skilled in the art from the descriptions herein that the novel sialocomplexes may be isolated and purified from a body fluid containing the sialocomplexes. In one illustration of this embodiment, peripheral blood was collected from a patient having SPMS and a pro-MS immune response, the blood was heparanized and centrifuged, and the plasma was harvested. The plasma was incubated under biological assay conditions with an affinity column comprising a matrix having immobilized thereto (using methods known on the art of affinity chromatography) one or more affinity ligands having binding specificity for the sialocomplexes. In a preferred embodiment, the affinity ligand immobilized to the affinity matrix comprised one or more of: an anti-anti-α(2,6)NeuAc Ab; and at least one antibody against at least one member of the sialoadhesin family. In a more preferred embodiment, the affinity ligand immobilized to the matrix is selected from the group consisting of anti-sTn mAb, anti-human MAG mAb, anti-human CD22 mAb, and a combination thereof. In illustrating this embodiment, an anti-sTn mAb was used as the immobilized affinity ligand in the affinity matrix. The affinity matrix comprised commercially available, activated beads (SEPHAROSE™) to which affinity ligand was immobilized according to the manufacturer's instructions. The column was equilibrated with a physiological buffer, and the plasma was then loaded onto, and chromato-

graphed through the column. The treated plasma (i.e., after being passed through the affinity column) was substantially free of detectable sialocomplexes comprising free sTn as determined by assaying a sample of the treated plasma in an immunoassay according to the present invention. In a plasma exchange procedure, the treated plasma may then be directed back into the individual using methods and instruments known to those skilled in the art of plasma exchange.

To isolate and purify the sialocomplexes bound to immobilized affinity ligand in the affinity column, the column was washed with buffer to remove any unbound plasma components, and then the sialocomplexes were eluted from the column using an elution buffer (e.g., 50 mM diethylamine pH 11.5, with 0.1% detergent (SDS)) for a sufficient time to elute the sialocomplexes, resulting in a preparation of isolated and purified sialocomplexes. Various other elution buffers and conditions will be apparent to those skilled in the art from the descriptions herein. If desired, the eluate may be extracted with a mixture of chloroform and methanol, or otherwise treated using methods known to those skilled in the art, to separate the glycolipid component of the sialocomplexes from other components of the sialo-complexes (e.g., one or more sialoadhesin family members). Analysis of the isolated and purified sialocomplexes was performed by polyacrylamide gel electrophoresis under non-reducing conditions, followed by Western Blot analysis. For example, anti-human MAG mAb and anti-sTn mAb were used separately as detection reagents (with a conjugate compri-sing anti-murine IgG labeled with horseradish peroxidase and a color development reagent comprising DAB) to detect the

sialocomplexes, wherein each detector molecule detected the
same band comprising the isolated and purified sialocom-
plexes. High performance thin layer chromatography using a
solvent system of chloroform: methanol:0.02% calcium
chloride (55:45:10) with 0.002% (w/v) of acridine orange to
stain glycolipid was used to show the presence of the
glycolipid component of the isolated and purified
sialocomplexes.

The isolated and purified sialocomplexes may be
used in a number of applications. For example, and as
apparent from the descriptions herein of immunoassay for-
mats, the isolated and purified sialocomplexes may be bound
to a solid support in forming a solid phase into which is
added a clinical sample to detect antibody or a soluble
sialoadhesin family member having binding specificity for
sialocomplexes that may be present in the clinical sample.
Alternatively, the isolated and purified sialocomplexes may
be used as an immunizing antigen to generate anti-
sialocomplexes antibodies (monoclonal or polyclonal)
utilizing standard techniques known in the art. Such
antibodies may be useful in an immunoassy format for
detecting sialocomplexes, and may be useful in an immuno-
affinity procedure to isolate and purify sialocomplexes.
More preferably, the isolated and purified sialocomplexes
may be quantitated, aliquoted in known amounts, and packaged
as a reference standard comprising a known amount of
sialocomplexes in the assay kit according to the present
invention.

The foregoing description of the specific embodiments of the present invention have been described in detail for purposes of illustration. In view of the descriptions and illustrations, others skilled in the art can, by applying, current knowledge, readily modify and/or adapt the present invention for various applications without departing from the basic concept, and therefore such modifications and/or adaptations are intended to be within the meaning and scope of the appended claims.

What is claimed is:

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1. An assay kit for use in analyzing a sample from an individual having, or suspected of having, a disease condition selected from the group consisting of multiple sclerosis ("MS"), a pro-MS immune response, and a combination thereof; the assay kit comprising a combination of two or more affinity ligands, wherein the affinity ligands are selected from the group consisting of an anti-human antibody, an affinity ligand having binding specificity for a member of the sialoadhesin family, and an affinity ligand having binding specificity for an epitope comprising a terminal alpha 2,6-linked sialic acid.

2. The assay kit according to claim 1, wherein at least one of the affinity ligands comprises a detection reagent.

3. The assay kit according to claim 2, wherein at least one of the affinity ligands comprising the detection reagent further comprises a detectable moiety.

4. The assay kit according to claim 1, wherein at least one of the affinity ligands comprises an affinity ligand immobilized to a solid phase.

5. The assay kit according to claim 1, wherein the anti-human antibody is selected from the group consisting of an anti-human IgG monoclonal antibody ("mAb"), an anti-human IgM mAb, and a combination thereof.

6. The assay kit according to claim 1, wherein the affinity ligand having binding specificity for an epitope comprising

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a terminal alpha 2,6-linked sialic acid comprises an anti-sTn mAb.

7.   The assay kit according to claim 1, wherein the affinity ligand having binding specificity for a member of the sialoadhesin family comprises an affinity ligand selected from the group consisting of an anti-human myelin-associated glycoprotein ("MAG") mAb, an anti-CD22 mAb, and a combination thereof.

8.   The assay kit according to claim 1, wherein the combination of two or more affinity ligands is a combination selected from the group consisting of an antibody which specifically binds an epitope comprising a terminal alpha 2,6-linked sialic acid ("anti-α(2,6)NeuAc Ab") and an anti-human IgG mAb, anti-sTn mAb and anti-human IgG mAb, anti-human MAG mAb and anti-human IgM mAb, anti-human MAG mAb and anti-human IgG mAb, anti-human MAG mAb and anti-α(2,6)NeuAc Ab, anti-human MAG mAb and anti-sTn mAb, anti-human MAG mAb and anti-human CD22 mAb, anti-human CD22 mAb and anti-human IgM mAb, anti-human CD22 mAb and anti-human IgG mAb, anti-human CD22 mAb and anti-α(2,6) NeuAc Ab, anti-human CD22 mAb and anti-sTn mAb, and a combination thereof.

9.   The assay kit according to claim 1, further comprising a reference standard comprising a known amount of sialocomplexes which are detectable by the combination of two or more affinity ligands.

10. A method for assaying a sample of an individual for an indicator of a disease condition selected from the group consisting of MS, a pro-MS immune response, and a combination thereof, the method comprising:

(a) admixing an aliquot of sample under biological assay conditions with a combination of two or more affinity ligands, wherein the two or more affinity ligands are selected from the group consisting of an anti-human antibody, an affinity ligand having binding specificity for a sialoadhesin family member, and an affinity ligand having binding specificity for an epitope comprising a terminal alpha 2,6-linked sialic acid, and wherein at least one of the affinity ligands comprises a detection reagent;

(b) measuring an amount of the detection reagent, if present, which is bound to the sample in determining a value of a marker in the sample;

(c) comparing the value of the marker determined to a comparative reference value;

wherein a difference in the value of the marker determined in the sample, when compared to the reference value, comprises an indicator of the presence of the disease condition.

11. The method according to claim 10, wherein the sample is selected from the group consisting of plasma, and serum.

12. The method according to claim 10, wherein at least one of the affinity ligands comprising the detection reagent further comprises a detectable moiety.

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13. The method according to claim 10, wherein at least one of the affinity ligands comprises an affinity ligand immobilized to a solid phase.

14. The method according to claim 10, wherein the anti-human antibody is selected from the group consisting of an anti-human IgG mAb, an anti-human IgM mAb, and a combination thereof.

15. The method according to claim 10, wherein the affinity ligand having binding specificity for an epitope comprising a terminal alpha 2,6-linked sialic acid comprises an anti-sTn mAb.

16. The method according to claim 10, wherein the affinity ligand having binding specificity for a member of the sialoadhesin family comprises an affinity ligand selected from the group consisting of an anti-human MAG mAb, an anti-CD22 mAb, and a combination thereof.

17. The method according to claim 10, wherein the combination of two or more affinity ligands is a combination selected from the group consisting of anti-α(2,6)NeuAc Ab and an anti-human IgG mAb, anti-sTn mAb and anti-human IgG mAb, anti-human MAG mAb and anti-human IgM mAb, anti-human MAG mAb and anti-human IgG mAb, anti-human MAG mAb and anti-α(2,6)NeuAc Ab, anti-human MAG mAb and anti-sTn mAb, anti-human MAG mAb and anti-human CD22 mAb, anti-human CD22 mAb and anti-human IgM mAb, anti-human CD22 mAb and anti-human IgG mAb, anti-human CD22 mAb and anti-α(2,6) NeuAc Ab,

anti-human CD22 mAb and anti-sTn mAb, and a combination thereof.

18. An indicator generated from the method according to claim 10.

19. A panel of indicators generated from the method according to claim 17.

20. A method for assaying a sample of an individual for monitoring the course of a disease condition selected from the group consisting of MS, a pro-MS immune response, and a combination thereof, the method comprising:
(a) admixing an aliquot of sample under biological assay conditions with a combination of two or more affinity ligands, wherein the two or more affinity ligands are selected from the group consisting of an anti-human antibody, an affinity ligand having binding specificity for a sialoadhesin family member, and an affinity ligand having binding specificity for an epitope comprising a terminal alpha 2,6-linked sialic acid, and wherein at least one of the affinity ligands comprises a detection reagent;
(b) measuring an amount of the detection reagent, if present, which is bound to the sample, in determining a value of a marker in the sample;
(c) comparing the value of the marker determined to a comparative value selected from the group consisting of a reference value, a baseline value, and a combination thereof;

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wherein a difference in the value of the marker determined in the sample, when compared to the comparative value, comprises an indicator of a change in course of the disease condition.

21. The method according to claim 20, wherein an indicator generated from the method may be used in a process selected from the group consisting of prognostically, for monitoring any effect of treatment on the course of the disease condition, and or for predicting a response of the disease condition to a therapeutic agent.

22. The method according to claim 20, wherein the sample is selected from the group consisting of plasma, and serum.

23. The method according to claim 20, wherein at least one of the affinity ligands comprising the detection reagent further comprises a detectable moiety.

24. The method according to claim 20, wherein at least one of the affinity ligands comprises an affinity ligand immobilized to a solid phase.

25. The method according to claim 20, wherein the anti-human antibody is selected from the group consisting of an anti-human IgG mAb, an anti-human IgM mAb, and a combination thereof.

26. The method according to claim 20, wherein the affinity ligand having binding specificity for an epitope comprising

a terminal alpha 2,6-linked sialic acid comprises an anti-sTn mAb.

27. The method according to claim 20, wherein the affinity ligand having binding specificity for a member of the sialoadhesin family comprises an affinity ligand selected from the group consisting of an anti-human MAG mAb, an anti-CD22 mAb, and a combination thereof.

28. The method according to claim 20, wherein the combination of two or more affinity ligands is a combination selected from the group consisting of anti-α(2,6)NeuAc Ab and an anti-human IgG mAb, anti-sTn mAb and anti-human IgG mAb, anti-human MAG mAb and anti-human IgM mAb, anti-human MAG mAb and anti-human IgG mAb, anti-human MAG mAb and anti-α(2,6)NeuAc Ab, anti-human MAG mAb and anti-sTn mAb, anti-human MAG mAb and anti-human CD22 mAb, anti-human CD22 mAb and anti-human IgM mAb, anti-human CD22 mAb and anti-human IgG mAb, anti-human CD22 mAb and anti-α(2,6) NeuAc Ab, anti-human CD22 mAb and anti-sTn mAb, and a combination thereof.

29. An indicator generated from the method according to claim 20.

30. A panel of indicators generated from the method acording to claim 28.

31. A method for assaying a sample of body fluid from an individual for sialocomplexes, the method comprising:

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(a) admixing an aliquot of the sample under biological assay conditions with a combination of two or more affinity ligands, wherein the two or more affinity ligands are selected from the group consisting of an anti-human antibody, an affinity ligand having binding specificity for a sialoadhesin family member, and an affinity ligand having binding specificity for an epitope comprising a terminal alpha 2,6-linked sialic acid, and wherein at least one of the affinity ligands comprises a detection reagent; and

(b) and measuring an amount of the detection reagent which is bound to sialocomplexes, if present, in determining an amount of the sialocomplexes.

32. The method according to claim 31, further comprising comparing the amount of sialocomplexes determined in the sample to a comparative value for the sialocomplexes, wherein the comparative value is selected from the group consisting of a reference value, a baseline value, and a combination thereof; wherein a difference in the amount of the sialocomplexes determined in the sample, when compared to the comparative value, comprises an indicator of a disease condition selected from the group consisting of MS, a pro-MS immune response, and a combination thereof.

33. The method according to claim 32, wherein an indicator generated from the method may be used in a process selected from the group consisting of prognostically, for monitoring any effect of treatment on the course of the disease condition, and or for predicting a response of the disease condition to a therapeutic agent.

34. The method according to claim 31, wherein the sample is selected from the group consisting of plasma, and serum.

35. The method according to claim 31, wherein at least one of the affinity ligands comprising the detection reagent further comprises a detectable moiety.

36. The method according to claim 31, wherein at least one of the affinity ligands comprises an affinity ligand immobilized to a solid phase.

37. The method according to claim 31, wherein the anti-human antibody is selected from the group consisting of an anti-human IgG mAb, an anti-human IgM mAb, and a combination thereof.

38. The method according to claim 31, wherein the affinity ligand having binding specificity for an epitope comprising a terminal alpha 2,6-linked sialic acid comprises an anti-sTn mAb.

39. The method according to claim 31, wherein the affinity ligand having binding specificity for a member of the sialoadhesin family comprises an affinity ligand selected from the group consisting of an anti-human MAG mAb, an anti-CD22 mAb, and a combination thereof.

40. The method according to claim 32, wherein the combination of two or more affinity ligands is a combination selected from the group consisting of anti-α(2,6)NeuAc Ab

and an anti-human IgG mAb, anti-sTn mAb and anti-human IgG mAb, anti-human MAG mAb and anti-human IgM mAb, anti-human MAG mAb and anti-human IgG mAb, anti-human MAG mAb and anti-α(2,6)NeuAc Ab, anti-human MAG mAb and anti-sTn mAb, anti-human MAG mAb and anti-human CD22 mAb, anti-human CD22 mAb and anti-human IgM mAb, anti-human CD22 mAb and anti-human IgG mAb, anti-human CD22 mAb and anti-α(2,6) NeuAc Ab, anti-human CD22 mAb and anti-sTn mAb, and a combination thereof.

41. An indicator generated from the method according to claim 32.

42. A panel of indicators generated from the method according to claim 40.

43. A method of removing sialocomplexes from the blood of an individual, the method comprising:

(a) collecting peripheral blood from the individual;

(b) separating plasma from the peripheral blood;

(c) treating the plasma by passing the plasma through an affinity column, wherein the affinity column comprises a matrix further comprising one or more affinity ligands immobilized thereto, wherein the one or more affinity ligands have binding specificity for the sialocomplexes, and wherein the one or more affinity ligands is an affinity ligand selected from the group consisting of anti-sTn mAb, anti-human MAG mAb, anti-human CD22 mAb, and a combination thereof.

44. The method of claim 43, further comprising eluting the sialocomplexes from the affinity matrix by contacting the affinity column with an elution buffer, resulting in a preparation comprised of isolated and purified sialocomplexes.

45. Isolated and purified sialocomplexes produced by the method according to claim 44.

ABSTRACT

Provided are methods, and assay kits, and compositions useful for generating an indicator of a disease condition selected from the group consisting of multiple sclerosis (MS), a pro-MS immune response, and a combination thereof. A method for assaying a sample of an individual for an indicator of the disease condition comprises contacting the sample with a combination of two or more affinity ligands, at least one of which comprises a detection reagent; measuring an amount of the detection reagent which is bound to the sample in determining a value of a marker in the sample; wherein a difference in the value of the marker determined in the sample, when compared to the reference value, comprises an indicator of the presence of the disease condition.
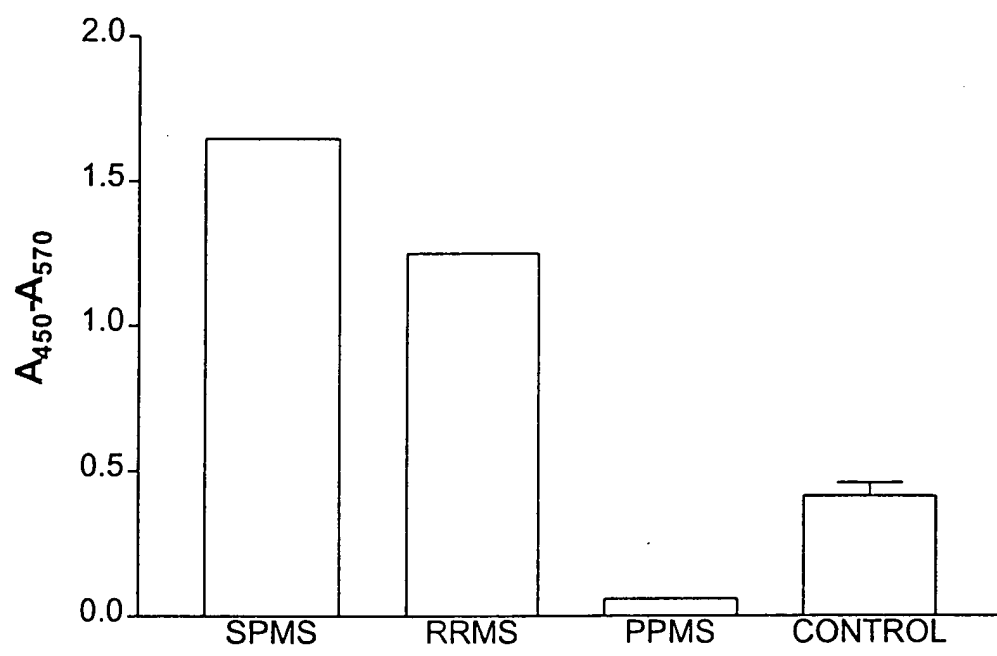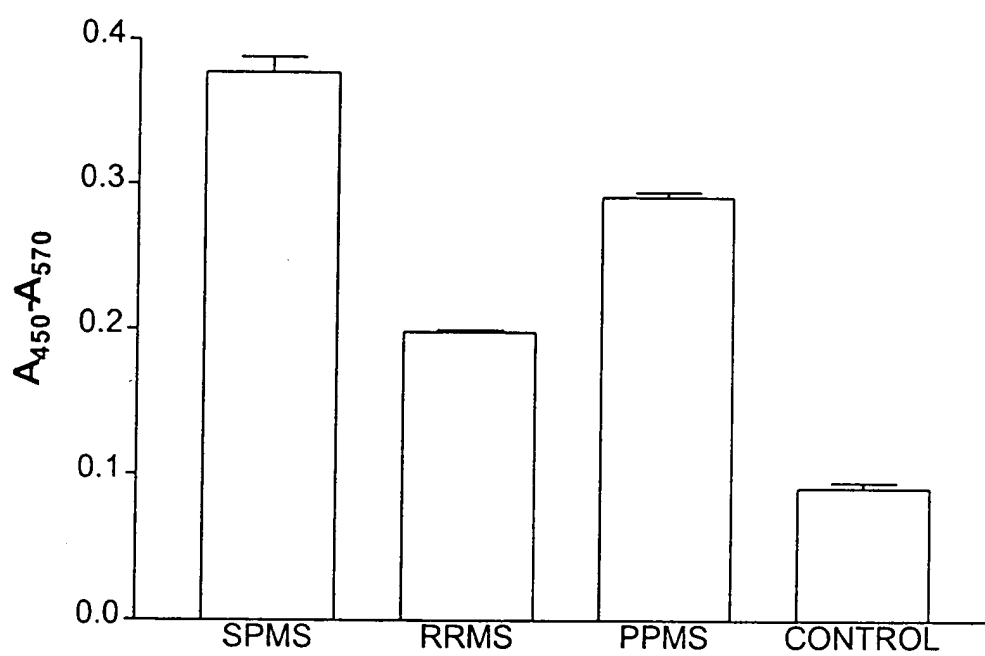
FIG.1

FIG. 2A
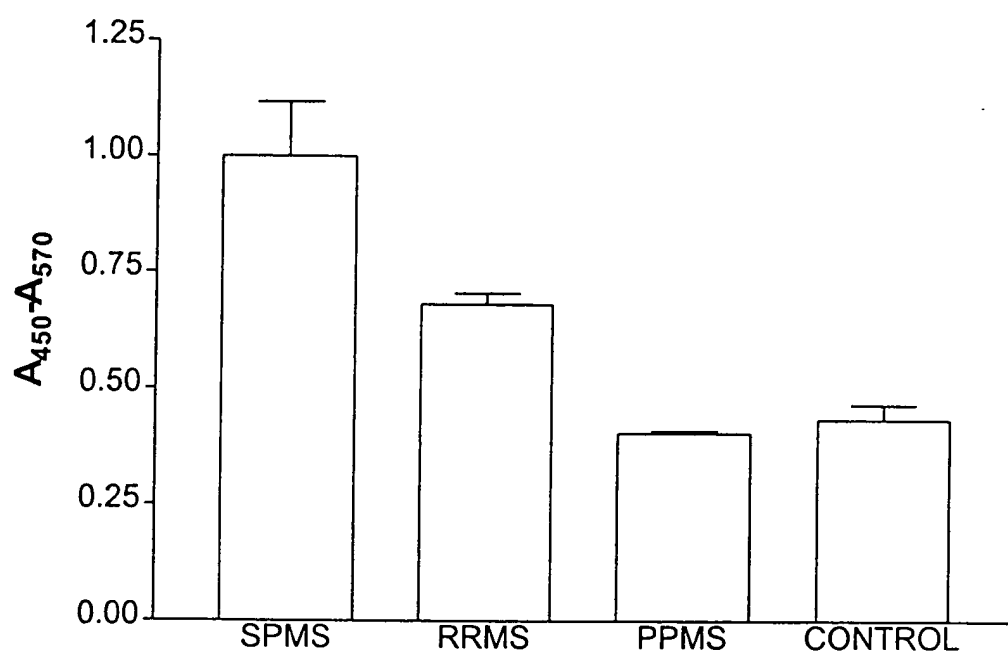
FIG. 2B
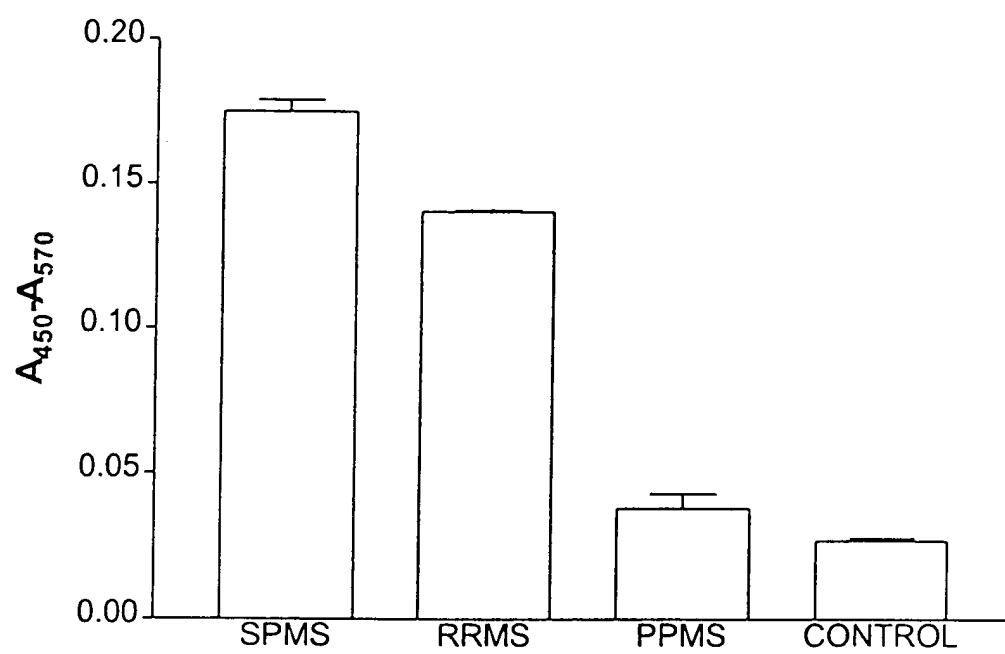
FIG. 2C
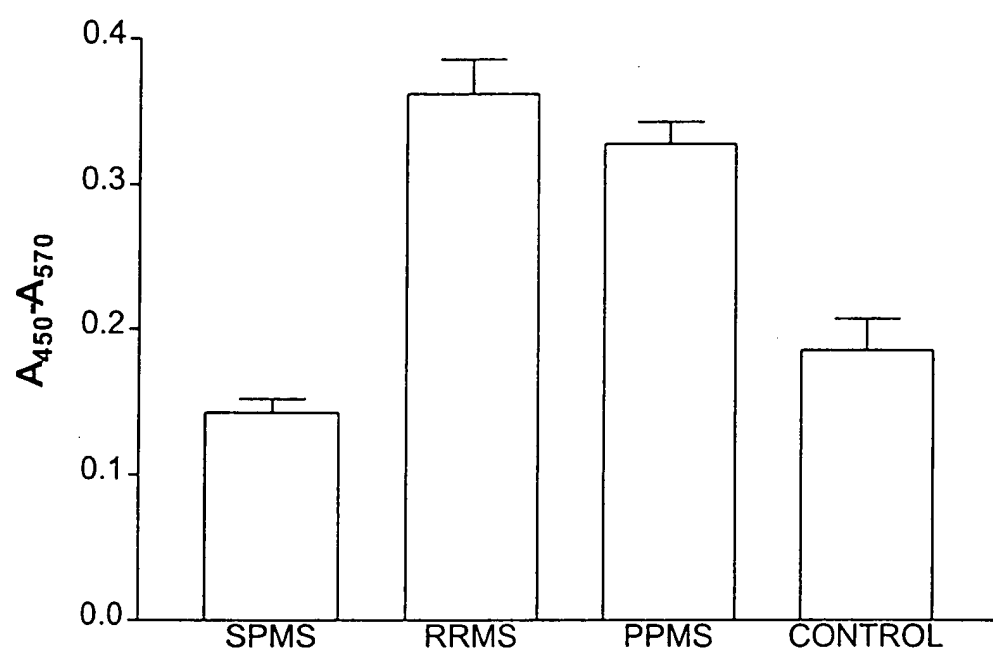
FIG. 3A
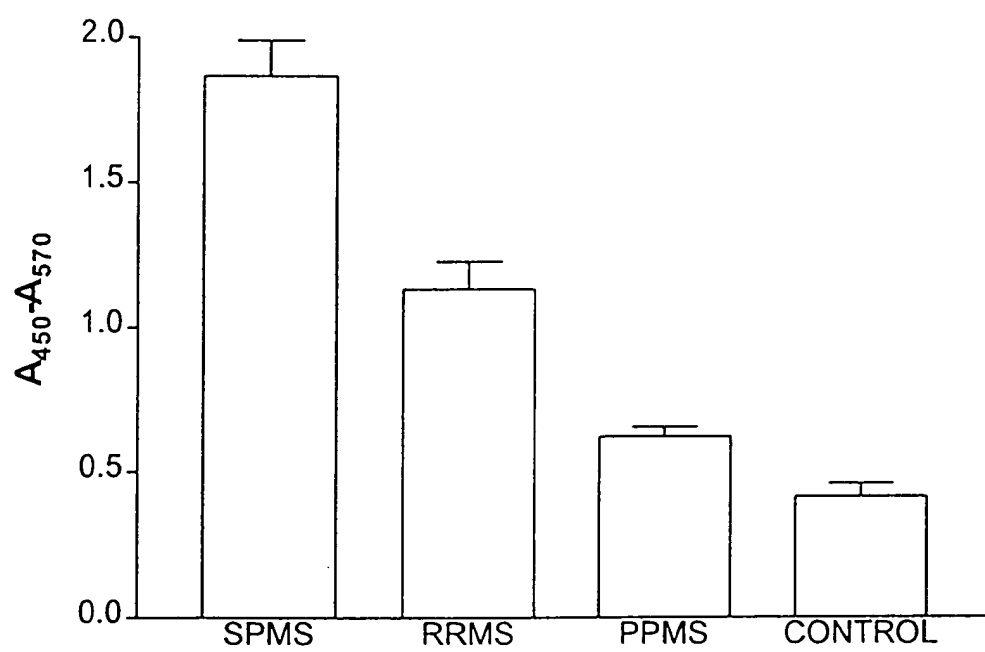
FIG. 3B
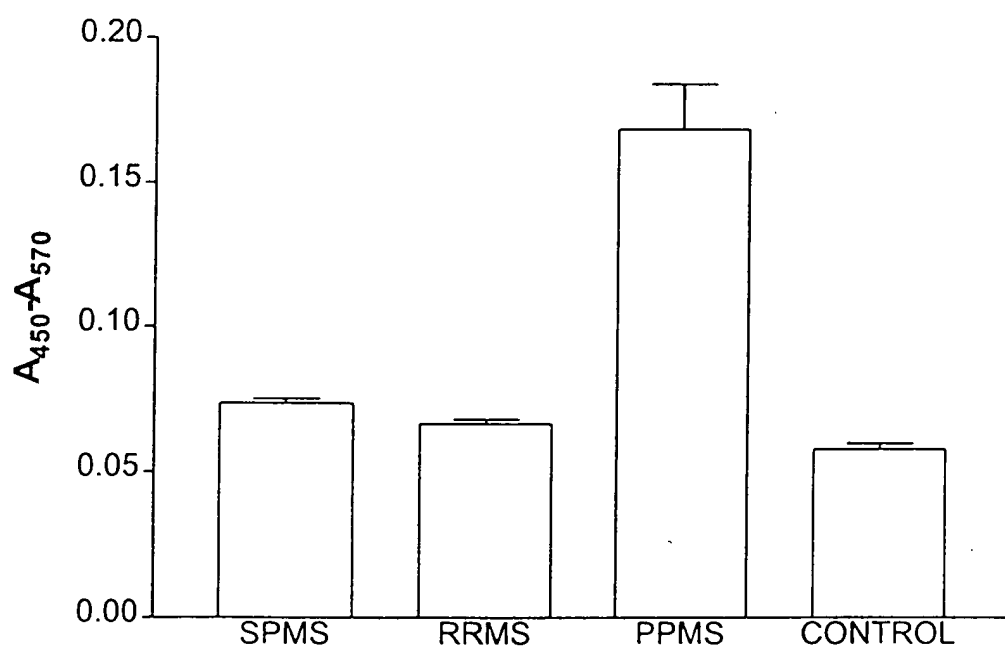
FIG. 3C
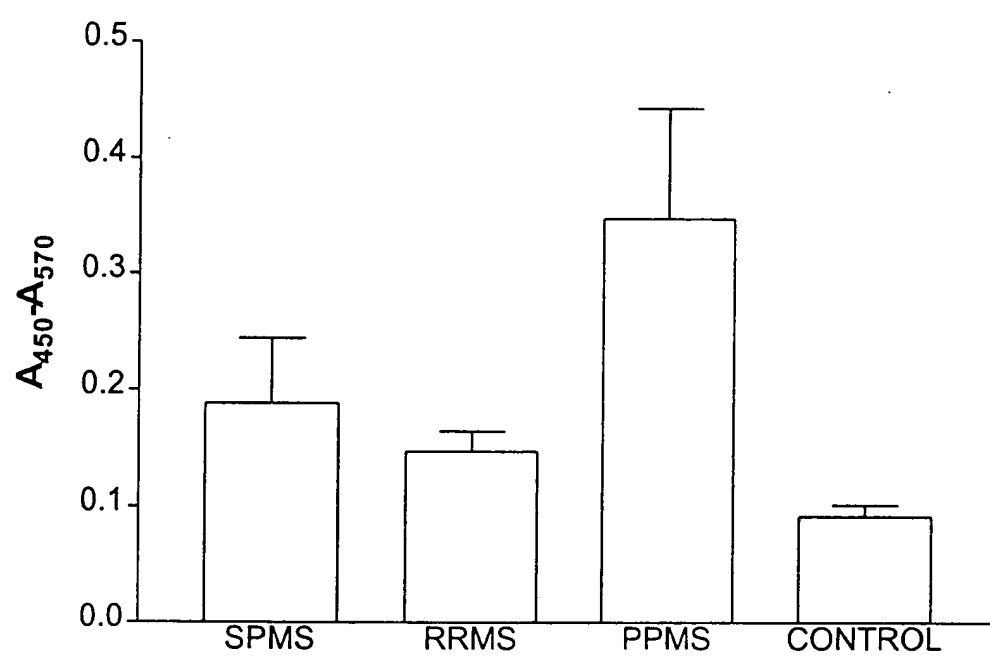
FIG. 3D

UNITED STATES PATENT AND TRADEMARK OFFICE

APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
09/643,277	08/22/2000	M. Bud Nelson	B-28	8660

21130 WSH. 7590 03/11/2003

BENESCH, FRIEDLANDER, COPLAN & ARONOFF LLP
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EXAMINER
SAUNDERS, DAVID A

ART UNIT	PAPER NUMBER
1644	

DATE MAILED: 03/11/2003

Please find below and/or attached an Office communication concerning this application or proceeding.

Office Action Summary	Application No. *103,377*	Applicant(s) *NELSON et al*
	Examiner *SAUNDERS*	Group Art Unit *1647*

—The MAILING DATE of this communication appears on the cover sheet beneath the correspondence address—

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE _____ MONTH(S) FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If the period for reply specified above is less than thirty (30) days, a reply within the statutory minimum of thirty (30) days will be considered timely.
- If NO period for reply is specified above, such period shall, by default, expire SIX (6) MONTHS from the mailing date of this communication .
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133).

Status

☑ Responsive to communication(s) filed on _12/2/02_ .

☑ This action is **FINAL.**

☐ Since this application is in condition for allowance except for formal matters, **prosecution as to the merits is closed** in accordance with the practice under *Ex parte Quayle,* 1935 C.D. 1 1; 453 O.G. 213.

Disposition of Claims

☑ Claim(s) _10-17 20-28, 31-40 46-62_ is/are pending in the application.

 Of the above claim(s) _____ is/are withdrawn from consideration.

☐ Claim(s)_____ is/are allowed.

☑ Claim(s) _10-17 20-28 31-40, 46-62_ is/are rejected.

☐ Claim(s)_____ is/are objected to.

☐ Claim(s)_____ are subject to restriction or election requirement.

Application Papers

☐ See the attached Notice of Draftsperson's Patent Drawing Review, PTO-948.

☐ The proposed drawing correction, filed on_____ is ☐ approved ☐ disapproved.

☐ The drawing(s) filed on_____ is/are objected to by the Examiner.

☐ The specification is objected to by the Examiner.

☐ The oath or declaration is objected to by the Examiner.

Priority under 35 U.S.C. § 119 (a)-(d)

☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 11 9(a)-(d).

 ☐ All ☐ Some* ☐ None of the CERTIFIED copies of the priority documents have been

 ☐ received.

 ☐ received in Application No. (Series Code/Serial Number)_____ .

 ☐ received in this national stage application from the International Bureau (PCT Rule 1 7.2(a)).

 *Certified copies not received:_____ .

Attachment(s)

☐ Information Disclosure Statement(s), PTO-1449, Paper No(s). _____

☐ Notice of Reference(s) Cited, PTO-892

☐ Notice of Draftsperson's Patent Drawing Review, PTO-948

☐ Interview Summary, PTO-413

☐ Notice of Informal Patent Application, PTO-152

☐ Other_____

Office Action Summary

Amendment of 12/2/02 has been entered.

Claims 11-17, 20-28, 31-40 and 46-52 are pending and under examination.

In claims 49 and 58 --immobilized-- has been misspelled. Appropriate correction is required.

Claims 11-17, 20-28, 31-40 and 46-52 are rejected under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention.

Claims 11-17, 20-28 and 31-40 are rejected under 112, second para. for all reasons previously stated in Paper 8.

New claims 46 and 54 correspond in their formatting to original claim 10. These are rejected for the reasons stated at pages 3-4 of Paper 8.

Applicant's response has done nothing but transverse and add new claims. As far as the examiner can ascertain from the response, applicant considers that the word "indicator" corresponds to what the examiner considered to be the "third interpretation" of this word. While applicant's response clarifies the record to some extent, it does not overcome all stated grounds of rejection. As noted, previously the claims themselves are so vague, that they fail to recite the proper functional relationship of all of the added reagents; hence amendment is required. See Paper 8, page 3, third and fourth paragraphs. Also, despite applicant's explanation of what "indicator" means, claim 10 remains confusing by virtue of reciting both "comparing" and "difference" in step (c). See Paper 8, page 4, second full paragraph.

Applicant's urgings filed on 12/2/02 have been considered but are unconvincing of patentability.

Substitute specification pages 17 and 18 have not been entered because applicant filed no marked up copies to show changes, 37 CFR 1.121.

Applicant's amendment necessitated the new ground(s) of rejection presented in this Office action. Accordingly, **THIS ACTION IS MADE FINAL.** See MPEP § 706.07(a). Applicant is reminded of the extension of time policy as set forth in 37 CFR 1.136(a).

A shortened statutory period for reply to this final action is set to expire THREE MONTHS from the mailing date of this action. In the event a first reply is filed within TWO MONTHS of the mailing date of this final action and the advisory action is not mailed until after the end of the THREE-MONTH shortened statutory period, then the shortened statutory period will expire on the date the advisory action is mailed, and any extension fee pursuant to 37 CFR 1.136(a) will be calculated from the mailing date of the advisory action. In no event, however, will the statutory period for reply expire later than SIX MONTHS from the date of this final action.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to David A. Saunders, Ph.D., whose telephone number is (703) 308-3976. The examiner can normally be reached on Monday-Thursday from 8:00 a.m. to 5:30 p.m. The examiner can also be reached on alternate Fridays.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's

supervisor, Christina Chan, can be reached on (703) 308-3973. The fax phone number for the

organization where this application or proceeding is assigned is (703) 308-4242.

Any inquiry of a general nature or relating to the status of this application or proceeding

should be directed to the receptionist whose telephone number is (703) 308-0196.

D. Saunders:jmr

March 03, 2003

DAVID SAUNDERS
PRIMARY EXAMINER
ART UNIT 182- / 1644

26983-850

UNITED STATES PATENT AND TRADEMARK OFFICE

APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
09/643,277	08/22/2000	M. Bud Nelson	B-28	8660

21130   7590   08/26/2002

BENESCH, FRIEDLANDER, COPLAN & ARONOFF LLP
ATTN: IP DEPARTMENT DOCKET CLERK
2300 BP TOWER
200 PUBLIC SQUARE
CLEVELAND, OH 44114

EXAMINER
SAUNDERS, DAVID A

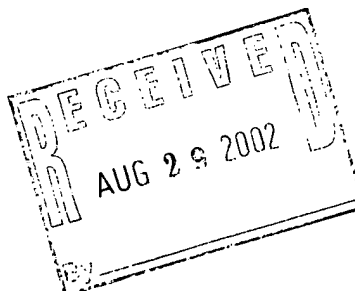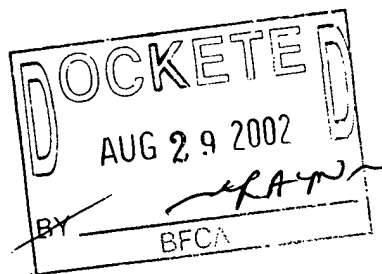| ART UNIT	PAPER NUMBER
1644	

DATE MAILED: 08/26/2002

Please find below and/or attached an Office communication concerning this application or proceeding.

DOCKETED
AUG 29 2002
BY
BFCA

RECEIVED
AUG 29 2002

PTO-90C (Rev. 07-01)

Office Action Summary	Application No. 043,277	Applicant(s) Nelson et al
	Examiner D. Saunders	Group Art Unit 1644

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE ___3___ MONTH(S) FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If the period for reply specified above is less than thirty (30) days, a reply within the statutory minimum of thirty (30) days will be considered timely.
- If NO period for reply is specified above, such period shall, by default, expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133).

Status

☑ Responsive to communication(s) filed on __4/15/02__ .

☐ This action is **FINAL.**

☐ Since this application is in condition for allowance except for formal matters, **prosecution as to the merits is closed** in accordance with the practice under *Ex parte Quayle,* 1935 C.D. 11; 453 O.G. 213.

Disposition of Claims

☑ Claim(s) __1-45__ is/are pending in the application.

Of the above claim(s) __1-9, 18-19, 29-30, 41-45__ is/are withdrawn from consideration.

☐ Claim(s)_____ is/are allowed.

☑ Claim(s) __10-17, 20-28, 31-40,__ is/are rejected.

☐ Claim(s)_____ is/are objected to.

☐ Claim(s)_____ are subject to restriction or election requirement.

Application Papers

☐ See the attached Notice of Draftsperson's Patent Drawing Review, PTO-948.

☐ The proposed drawing correction, filed on_____ is ☐ approved ☐ disapproved.

☐ The drawing(s) filed on_____ is/are objected to by the Examiner.

☐ The specification is objected to by the Examiner.

☐ The oath or declaration is objected to by the Examiner.

Priority under 35 U.S.C. § 119 (a)-(d)

☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d).

☐ All ☐ Some* ☐ None of the CERTIFIED copies of the priority documents have been

☐ received.

☐ received in Application No. (Series Code/Serial Number)_____

☐ received in this national stage application from the International Bureau (PCT Rule 17.2(a)). AUG 29 2002

*Certified copies not received:_____

RECEIVED AUG 29 2002

DOCKETED AUG 29 2002 BY _____ BFCA

Attachment(s)

☑ Information Disclosure Statement(s), PTO-1449, Paper No(s). _____

☑ Notice of Reference(s) Cited, PTO-892

☐ Notice of Draftsperson's Patent Drawing Review, PTO-948

☐ Interview Summary, PTO-413

☐ Notice of Informal Patent Application, PTO-152

☐ Other_____

Office Action Summary

The claims pending are 1-45.

Applicant's election with traverse of Group II (claims 10-17, 20-28 and 31-40) in Paper

No. 5 (filed 4/15/02) is acknowledged. The traversal is on the ground(s) that no undue burden

would be required to search for the additional invention and species. This is not found

persuasive because the search for the kits of Group I would be more extensive, because the

components of the kits could be used to test for conditions other than MS (e.g. for immune

complexes of shed tumor antigens in cancer patients). The search areas and terms for the method

Group I (removing sialo complexes) would be completely different from that for the assay. The

search for the sialo complexes of Group IV would require a search of extensive prior art

unrelated to MS (e.g. sialo complexes exist in cancer patients). The examiner does not even

know what the "indicators" of Group III are (see 112, second infra) and cannot comment except

that these appear to be in a different statutory class (or even a nonstatutory class) from the

method and searches for these would be more extensive.

The requirement is still deemed proper and is therefore made FINAL.

Among the claims of Group II, claims 10-15, 17, 20-26, 28, 31-38 and 39-40 read on the

elected species.

The drawings of 8/22/00 have been approved by the draftsman.

The disclosure is objected to because of the following informalities: at page 17 there is an

extraneous dashed line.

Appropriate correction is required.

At page 18, the current status of the referenced copending application must be updated.

Claims 10-17, 20-28 and 31-40 are rejected under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention.

Claims 10 and 20 are confusing as to what the recited "marker" is. It is not clear if the marker, which is determined by measuring the amount of the detection reagent, is present in complexes (of the two binding partners for the two affinity ligands employed in step (a) or is simply the binding partner of the affinity ligand that comprises a detection reagent. Note that claims 10 and 20 fail to state any function of the affinity ligand having no detection reagent in steps (b) or (c).

In like manner claim 31 fails to state, in step (b), how the antibody which does not comprise a detection reagent (added in step (a) is involved in determining the amount of the sialocomplexes.

In claims 10, 20, and 32 the term "indicator" is unclear. For example, is this the same as the "marker" (complexes present in the sample); is this a detectable entity (such as a colored solution in a test well) generated when measuring the amount of detection reagent; or is this a non-physical entity (such as a numerial valve obtained by subtraction to obtain the difference in the value of the marker in the sample and the reference value)?

Several of the specification pages recite "the markers comprise indicators." (e.g. pages 16 and 19-21). These teachings would suggest the first of the above noted possible interpretations

of the word "indicator" is correct. If so use of both the word "marker" and "indicator" is confusing, and applicant must use one term consistently.

Non-elected claims 18-19 and 29-30 refer to "indicator(s) generated from the method according to...". This would suggest that the second of the above interpretations of the word "indicator" is intended.

Claims 10, 20 and 32 themselves and the specification Examples would suggest that the "indicator" is some sort of numerical value. This would be consistent with the third of the above interpretations of the word "indicator". If this is intended, the claim must be further clarified as to how this indicator value is obtained. For example "difference" implies a substraction, while "comparing" implies a division to obtain a quotient.

Clarification of the claims and an explanation of what is intended is required.

The following references are cited as of interest.

Hakamori et al., Kannagi et al., and Yamashima show various monoclonal antibodies directed against carbohydrate epitopes containing an alpha 2, 6 linked siayl moiety. None of these references teaches detection of the target antigen in an immune complex or a sialoadhesin complex. Shimada et al. show an antibody to BDIa (TABLE 3 shows this does not have terminal 2-6 linkages). They measure GDIa in immune complexes by an assay format that substantially differs fronm that instantly.

Collins shows a sandwich assay for measuring a complex of an antigen and a host (patient) antibody thereto. Collins does not teach measuring such a complex in MS patients.

Claims 10-17, 20-28 and 31-40 are allowable over the prior art of record, given the

definition of the term "sialocomplexes" at specification page 6.

Any inquiry concerning this communication or earlier communications from the

examiner should be directed to David A. Saunders, Ph.D., whose telephone number is (703) 308-

3976. The examiner can normally be reached on Monday-Thursday from 8:00 a.m. to 5:30 p.m.

The examiner can also be reached on alternate Fridays.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's

supervisor, Christian Chan, can be reached on (703) 308-3973. The fax phone number for the

organization where this application or proceeding is assigned is (703) 308-4242.

Any inquiry of a general nature or relating to the status of this application or proceeding

should be directed to the receptionist whose telephone number is (703) 308-0196.

D. Saunders:jmr

August 8, 2002

DAVID SAUNDERS
PRIMARY EXAMINER
ART UNIT 182   1644

INFORMATION DISCLOSURE CITATION

(Use several sheets if necessary)

Docket Number (Optio	Application Number
B-28	09/643,277

Applicant(s)
Nelson and Barbera-Guillem

Filing Date	Group Art Unit
22 August 2000	1644

U.S. PATENT DOCUMENTS

*EXAMINER INITIAL	REF	DOCUMENT NUMBER	DATE	NAME	CLASS	SUBCLASS	FILING DATE IF APPROPRIATE
DUS		6,080,554	06/27/2000	Campine	435	7.1	

FOREIGN PATENT DOCUMENTS

	REF	DOCUMENT NUMBER	DATE	COUNTRY	CLASS	SUBCLASS	Translation YES	NO

OTHER DOCUMENTS *(Including Author, Title, Date, Pertinent Pages, Etc.)*

dus	Sadatipour et al., 1998, Increased circulating antiganglioside antibodies in primary and secondary progressive multiple sclerosis, Annals of Neurology, 44:980-983.
dus	Acarin et al., 1996, Different antiganglioside antibody pattern between relapsing-remitting and progressive multiple sclerosis, Acta Neurol. Scand., 93:99-103.
dus	Barbera-Guillem et al., 1999, Promotion of tumor invasion by cooperation of granulocytes and macrophages activated by anti-tumor antibodies, Neoplasia, 1:453-460.

EXAMINER David U Saunders	DATE CONSIDERED 7/26/02

EXAMINER: Initial if citation considered, whether or not citation is in conformance with MPEP Section 609; Draw line through citation if not in conformance and not considered. Include copy of this form with next communication to applicant.

F rm PTO-A820
(als form PTO-1449)

P09A/REV04

Patent and Trademark Office * U.S. DEPARTMENT OF COMMERCE

SHEET   OF

FORM PTO-892 (REV. 2-92)	U.S. DEPARTMENT OF COMMERCE PATENT AND TRADEMARK OFFICE	SERIAL NO. 64-3,277	GROUP ART UNIT 1644	ATTACHMENT TO PAPER NUMBER	8
NOTICE OF REFERENCES CITED		APPLICANT(S) NILSON et al			

U.S. PATENT DOCUMENTS

*		DOCUMENT NO.	DATE	NAME	CLASS	SUB-CLASS	FILING DATE IF APPROPRIATE
	A	5 0 1 1 9 2 0	4/1991	HAKOMORI et al	536	53	
	B	5 1 7 3 4 2 0	12/1992	KANNAGI et al	435	240.27	
	C	5 1 9 2 6 6 2	3/1993	SHIMADA et al	435	7.23	
	D	5 1 9 4 3 8 5	3/1993	YAMASHIMA	435	240.27	
	E	6 3 8 3 7 4 0	5/2002	COLLINS	435	5	
	F						
	G						
	H						
	I						
	J						
	K						

FOREIGN PATENT DOCUMENTS

*		DOCUMENT NO.	DATE	COUNTRY	NAME	CLASS	SUB-CLASS	PERTINENT SHTS. DWG	PP. SPEC.
	L								
	M								

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant: Nelson   Examiner: Saunders, D. A.

Serial No.: 09/643,277   Art Unit: 1644

Filing Date: August 22, 2000

Title: **COMPOSITIONS, ASSAY KITS AND METHODS FOR USE RELATED TO A DISEASE CONDITION COMPRISING MULTIPLE SCLEROSIS AND/OR A PRO-MS IMMUNE RESPONSE**

Docket No.: B-28 (26983-50)

Commissioner for Patents
Washington, D.C. 20231

AMENDMENT A AND RESPONSE TO OFFICE ACTION

Dear Sir:

This is in response to the Office Action dated August 26, 2002, issued in connection with the above-referenced application. The Office Action set a three-month statutory period to respond. Accordingly, this response is timely filed.

Please amend the subject application as follows:

IN THE CLAIMS:

In view of the Examiner's position in the restriction requirement, please cancel claims 1-9, 18, 19, 29-30 and 41-45 without prejudice for reintroduction in this or a later filed application.

Please add new claims 46-62 as follows.

46.　(New) A method comprising:

(a) admixing an aliquot of sample under biological assay conditions with a combination of two or more affinity ligands, wherein the affinity ligands are selected from the group consisting of an anti-human antibody, an affinity ligand having binding specificity for a sialoadhesin family member, and an affinity ligand having binding specificity for an epitope comprising a terminal alpha 2,6-linked sialic acid, and wherein at least one of the affinity ligands comprises a detection reagent;

(b) measuring an amount of the detection reagent which is bound to the sample to determine a value of a marker in the sample;

(c) comparing the value of the marker in the sample to a comparative reference value;

wherein the comparing indicates the presence or absence of a disease condition.

47.　(New) The method according to claim 46, wherein the sample is selected from the group consisting of plasma, and serum.

48.　(New)　The method according to claim 46, wherein at least one of the affinity ligands comprising the detection reagent further comprises a detectable moeity.

49.　(New)　The method according to claim 46, wherein at least one of the affinity ligands comprises an affinity ligand immobolized to a solid phase.

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50. (New) The method according to claim 46, wherein the anti-human antibody is selected from the group consisting of an anti-human IgG mAb, an anti-human IgM mAb, and a combination thereof.

51. (New) The method according to claim 46, wherein the affinity ligand having binding specificity for an epitope comprising a terminal alpha 2,6-linked sialic acid comprises an anti-sTn mAb.

52. (New) The method according to claim 46, wherein the affinity ligand having binding specificity for a member of the sialoadhesin family comprises an affinity ligand selected from the group consisting of an anti-human MAG mAb, an anti-CD22 mAb, and a combination thereof.

53. (New) The method according to claim 46, wherein the combination of two or more affinity ligands is a combination selected from the group consisting of anti-α(2,6) NeuAc Ab and an anti-human IgG mAb, anti-sTn mAb and anti-human IgG mAb, anti-human MAG mAb and anti-human IgM mAb, anti-human MAG mAb and anti-human IgG mAb, anti-human MAG mAb and anti-α(2,6) NeuAc Ab, anti-human MAG mAb and anti-sTn mAb, anti-human MAG mAb and anti-human CD22mAb, anti-human CD22 mAb and anti-human IgM mAb, anti-human CD22 mAb and anti-human IgG mAb, anti-human CD22 mAb and anti-α(2,6) NeuAc Ab, anti-human CD22 mAb and anti-sTn mAb, and a combination thereof.

54.   (New) A method comprising:

(a) admixing an aliquot of sample under biological assay conditions with a combination of two or more affinity ligands, wherein the affinity ligands are selected from the group consisting of an anti-human antibody, an affinity ligand having binding specificity for a sialoadhesin family member, and an affinity ligand having binding specificity for an epitope comprising a terminal alpha 2,6-linked sialic acid, and wherein at least one of the affinity ligands comprises a detection reagent;

(b) determining a level of the detection reagent which is bound to the sample;

(c) comparing the level of the detection reagent to a comparative reference;

(d) deriving an indicator for the presence or absence of a disease condition selected form the group consisting of MS, a pro-MS immune response, and a combination thereof based on the comparing.

55.   (New) The method according to claim 54, wherein the indicator may be used in a process selected from the group consisting of prognostically, for monitoring any effect of treatment on the course of the disease condition, and or for predicting a response of the disease condition to a therapeutic agent.

56.   (New) The method according to claim 54, wherein the sample is selected from the group consisting of plasma, and serum.

57.   (New) The method according to claim 54, wherein at least one of the affinity ligands comprising the detection reagent further comprises a detectable moeity.

58.   (New) The method according to claim 54, wherein at least one of the affinity ligands comprises an affinity ligand immobolized to a solid phase.

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59.   (New)   The method according to claim 54, wherein the anti-human antibody is selected from the group consisting of an anti-human IgG mAb, an anti-human IgM mAb, and a combination thereof.

60.   (New)   The method according to claim 54, wherein the affinity ligand having binding specificity for an epitope comprising a terminal alpha 2,6-linked sialic acid comprises an anti-sTn mAb.

61.   (New)   The method according to claim 54, wherein the affinity ligand having binding specificity for a member of the sialoadhesin family comprises an affinity ligand selected from the group consisting of an anti-human MAG mAb, an anti-CD22 mAb, and a combination thereof.

62.   (New) The method according to claim 54, wherein the combination of two or more affinity ligands is a combination selected from the group consisting of anti-α(2,6) NeuAc Ab and an anti-human IgG mAb, anti-sTn mAb and anti-human IgG mAb, anti-human MAG mAb and anti-human IgM mAb, anti-human MAG mAb and anti-human IgG mAb, anti-human MAG mAb and anti-α(2,6) NeuAc Ab, anti-human MAG mAb and anti-sTn mAb, anti-human MAG mAb and anti-human CD22mAb, anti-human CD22 mAb and anti-human IgM mAb, anti-human CD22 mAb and anti-human IgG mAb, anti-human CD22 mAb and anti-α(2,6) NeuAc Ab, anti-human CD22 mAb and anti-sTn mAb, and a combination thereof.

IN THE SPECIFICATION:

The extraneous line on page 18 has been removed. A substitute sheet is attached .

At page 17, the current status of the copending application has been provided. Again a substitute sheet is attached.

REMARKS

Applicant wishes to thank the Examiner for the careful consideration given this case. As examined, claims 1-45 were pending. Claims 1-9, 18-19, 29-30, and 41-45 were subjected to restriction as being drawn to a non-elected group. Accordingly those claims have been canceled by the present amendment. New claims 46-62 have been added. Thus, claims 10-17, 20-28, 31-40 and 46-62 are presented for further consideration in light of the remarks below.

THE EXAMINER'S ACTION

In the Office Action mailed August 26, 2002, the Examiner made final the restriction requirement.

The Examiner also objected to the specification.

The Examiner also rejected claims 10-17, 20-28 and 31-40 under 35 U.S.C. 112, Second Paragraph as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention. Specifically, the Examiner found the term "marker" in claims 10 and 20, as well as the term "indicator" in claims 10, 20 and 32 unclear.

REJECTIONS UNDER 35 U.S.C. § 112, Second Paragraph

The marker as used in the claims is the entity being measured and compared against a reference value to determine the presence or absence of disease. As illustrated in Example 1, the value of the marker, which comprised

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human IgG with a sTn epitope, was measured and compared to the reference value. To determine the value, or amount, of the marker, one may measure the bound detection reagent, which consists of an affinity ligand, which has affinity and specificity for sialocomplexes, and a detectable moeity bound to the sialocomplex. The language "if present" is to modify "an amount of detection reagent which is bound to the sample." If there is no detection reagent bound to the sample, then the lack of binding indicates the absence of sialocomplexes.

Indicator, as used in the claims, refers to markers meeting a condition. The condition may be comparing a reference value to the measured value of a marker. The comparison entails determining a difference between the two values, the difference (or similarity) can be obtained through subtraction or division, or any other method which would show a difference or lack thereof.

For improved clarity, attention is directed to the specification at page 15, line 27 through page 17, line 4.

The Examiner will appreciate that newly added claims 46-62 recite elements previously found allowable. Accordingly, entry and allowance are not believed to enter new matter or necessitate a new search.

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CONCLUSION

In light of the remarks presented above, Applicant respectfully submits that the present application is in condition for allowance and notice to such effect is respectfully requested.

The Commissioner is hereby authorized to charge any additional fees, or credit any overpayment, to Deposit Account No. 02-2051 identifying Attorney Docket No. 26983-50.

Respectfully submitted,

Dated: 11/25/02   By: _____

W. Scott Harders
Reg. No. 42,629

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 COPLAN & ARONOFF LLP
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200 Public Square
Cleveland, Ohio 44114
(216) 363-4443

condition selected from the group consisting of MS, a pro-MS immune response, and a combination thereof; in providing an additional parameter to a competent health professional in making a medical opinion.

The term "pro-MS immune response" is used herein, for purposes of the specification and claims, to mean a humoral immune response induced against an epitope comprising a terminal alpha 2,6-linked sialic acid (e.g., comprising sialyl Tn or sTn which comprises a terminal sialic acid alpha 2,6-linked to GalNac) of a shed antigen (glyco-molecule), resulting in production of IgG antibody against the epitope ("anti-α(2,6)NeuAc Ab"), and complexes comprised of the shed antigen comprising the epitope complexed to anti-α(2,6)NeuAc Ab, and may further comprise one or more members of the sialoadhesin family; wherein the shed antigen is released or produced particularly in relation to CNS tissue damage characteristic of MS during the MS disease process. In a preferred embodiment, the resultant complexes bind to and induce Fc receptor-expressing cells (e.g., one or more cell types selected from the group consisting of granulocytes, macrophages, microglia, activated mast cells, astrocytes, oligodendrocytes) which results in the release of inflammatory mediators (e.g., cytokines and/or tissue degradative enzymes) which may promote (contribute to) CNS tissue damage characteristic of MS (e.g., demyelination and plaques characteristic of MS). A similar immune response, a pro-tumor response, has been described in U.S. Patent No. 6,251,616 (the disclosure of which is herein incorporated by reference). In a preferred embodiment, the anti-α(2,6) NeuAc Ab is induced by a shed

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antigen comprising glycolipid, as previously described herein in more detail. Cellular markers for a pro-MS immune response have been described in detail in U.S. application Serial No. 60/150256, now abandoned, the disclosure of which is herein incorporated by reference.

The term "biological assay conditions" is used herein, for purposes of the specification and claims, to mean those conditions under which an affinity ligand can specifically bind to the molecule for which it has binding specificity (e.g., a component of the sialocomplexes according to the present invention). As known to those skilled in the art, such conditions may include one or more of: a pH range of from about 5 to 9, ionic strengths such as that ranging from distilled water to about 1 molar sodium chloride, and a temperature in the range of from about 4°C to about 45°C; and may further include a time sufficient for binding to occur (e.g., in a range of from about 10 minutes to about 2 hours).

There is a need for clinical tests which can aid in the diagnosis of MS. Additionally, there is a need for clinical tests which can detect one or more of: individuals at risk of developing MS, initiation of inflammatory processes preceding development of a pattern of clinical symptoms characteristic of MS, detection of MS at an earlier point in time than currently available techniques, and monitoring the progression of MS (e.g., from RRMS to SPMS). Further, presently there are no commercially available tests to evaluate for the presence of a pro-MS immune response. There is a need for laboratory tests that distinguish

APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
09/643,277	08/22/2000	M. Bud Nelson	B-28	8660

21130   7590   07/18/2003

BENESCH, FRIEDLANDER, COPLAN & ARONOFF LLP
ATTN: IP DEPARTMENT DOCKET CLERK
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CLEVELAND, OH 44114

26 983 -50

EXAMINER
SAUNDERS, DAVID A

ART UNIT	PAPER NUMBER
1644	

DATE MAILED: 07/18/2003

Please find below and/or attached an Office communication concerning this application or proceeding.

	Application No.	Applicant(s)
Advisory Action	09/643,277	NELSON ET AL.
	Examin r	Art Unit
	David A Saunders, PhD	1644

--Th MAILING DATE of this communication appears on the cover sheet with th correspondence address --

THE REPLY FILED 5/15/03 FAILS TO PLACE THIS APPLICATION IN CONDITION FOR ALLOWANCE.
Therefore, further action by the applicant is required to avoid abandonment of this application. A proper reply to a final rejection under 37 CFR 1.113 may <u>only</u> be either: (1) a timely filed amendment which places the application in condition for allowance; (2) a timely filed Notice of Appeal (with appeal fee); or (3) a timely filed Request for Continued Examination (RCE) in compliance with 37 CFR 1.114.

PERIOD FOR REPLY [check either a) or b)]

a) ☒ The period for reply expires <u>3</u> months from the mailing date of the final rejection.

b) ☐ The period for reply expires on: (1) the mailing date of this Advisory Action, or (2) the date set forth in the final rejection, whichever is later. In no event, however, will the statutory period for reply expire later than SIX MONTHS from the mailing date of the final rejection.
ONLY CHECK THIS BOX WHEN THE FIRST REPLY WAS FILED WITHIN TWO MONTHS OF THE FINAL REJECTION. See MPEP 706.07(f).

Extensions of time may be obtained under 37 CFR 1.136(a). The date on which the petition under 37 CFR 1.136(a) and the appropriate extension fee have been filed is the date for purposes of determining the period of extension and the corresponding amount of the fee. The appropriate extension fee under 37 CFR 1.17(a) is calculated from: (1) the expiration date of the shortened statutory period for reply originally set in the final Office action; or (2) as set forth in (b) above, if checked. Any reply received by the Office later than three months after the mailing date of the final rejection, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

1. ☐ A Notice of Appeal was filed on _____. Appellant's Brief must be filed within the period set forth in 37 CFR 1.192(a), or any extension thereof (37 CFR 1.191(d)), to avoid dismissal of the appeal.

2. ☐ The proposed amendment(s) will not be entered because:

    (a) ☐ they raise new issues that would require further consideration and/or search (see NOTE below);

    (b) ☐ they raise the issue of new matter (see Note below);

    (c) ☐ they are not deemed to place the application in better form for appeal by materially reducing or simplifying the issues for appeal; and/or

    (d) ☐ they present additional claims without canceling a corresponding number of finally rejected claims.
       NOTE: _____.

3. ☐ Applicant's reply has overcome the following rejection(s): _____.

4. ☐ Newly proposed or amended claim(s) _____ would be allowable if submitted in a separate, timely filed amendment canceling the non-allowable claim(s).

5. ☒ The a)☐ affidavit, b)☐ exhibit, or c)☒ request for reconsideration has been considered but does NOT place the application in condition for allowance because: *See Continuation Sheet.*

6. ☐ The affidavit or exhibit will NOT be considered because it is not directed SOLELY to issues which were newly raised by the Examiner in the final rejection.

7. ☒ For purposes of Appeal, the proposed amendment(s) a)☐ will not be entered or b)☒ will be entered and an explanation of how the new or amended claims would be rejected is provided below or appended.

    The status of the claim(s) is (or will be) as follows:

    Claim(s) allowed: _____.

    Claim(s) objected to: _____.

    Claim(s) rejected: *11-17,20-28,31-40 and 46-62.*

    Claim(s) withdrawn from consideration: _____.

8. ☐ The proposed drawing correction filed on _____ is a)☐ approved or b)☐ disapproved by the Examiner.

9. ☐ Note the attached Information Disclosure Statement(s)(PTO-1449) Paper No(s). _____.

10. ☐ Other: _____.

Continuation of 5. does NOT place the application in condition for allowance because: Applicant's response has clarified the record as to what he intends to claim by reciting "indicator". For what applicant intends, the term --indication-- would be more appropriate than "indicator" in claims 10, 20, 32 and 54. The term "indication" is conventionally used in claim language for clinical diagnostic methods, in a concluding step that relates the presence/amount of a diagnostic marker to the presence/absence/stage/course of a disease condition. The term "indicator", on the other hand, implies a chemical entity which provides a signal response in an assay. The fact that applicant used the term "indicator" as an entity to be claimed (nonelected claims 18-19, 29-30, 41-42) implied to the examiner that the term "indicator meant some physical/chemical entity and led to confusion in examination of elected claims.

Claims 10, 20, 31, 46 and 54 remain confusing by failing to recite how the affinity ligand which does not comprise a detection reagent participates to form any sort of complex, to which the affinity ligand which comprises a detection reagent becomes bound (Nothing even recites that a complex including these two affinity ligands is formed). While applicant's urgings may be enlightening, the claims per se must clearly define the cooperative relationship of all of the recited reagents, without which there is a gap in the claimed method steps.

Regarding the terms "comparing" and "difference", the examiner concurs that these need not be interpreted in any mathematical sense.

DAVID SAUNDERS
PRIMARY EXAMINER
ART UNIT 182 / 6+4